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LABORATORY MANUAL
FOR
GENERAL ZOOLOGY

BY

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and
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LABORATORY MANUAL FOR GENERAL ZOOLOGY

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PREFACE

This manual is designed for the general introductory course in zoology in college or university. It includes some general instructions for students, detailed exercises on the structure and physiology of the frog, others on the general principles of animal biology, and a series on common representatives of the principal groups of invertebrates. Each exercise gives detailed instructions to guide the student in making dissections and carrying through other laboratory procedures, and also includes questions to focus attention on the important observations and conclusions to be made with each type of material. Other questions deal with related subjects or animals, including some practical topics. Descriptions and illustrations of the various animal forms, and other information about them, together with discussions of general zoological principles, will be found in the author's textbook, "General Zoology" (McGraw-Hill Book Company, Inc.).

The manual follows somewhat the plan of the text and affords a fair coverage for general zoology. More exercises are included than can be dealt with in two semesters of 12 to 16 weeks. Each exercise is confined to one subject or animal type; some are short and others will require two (or more) laboratory periods of 2 or 3 hours to complete. Each is arranged and subdivided so that an instructor may choose the portions suited to his own viewpoint and local conditions. The extent and variety of material included will permit considerable latitude in outlining a laboratory program. With appropriate selection, emphasis may be placed either on general principles or on animal types. The sequence of exercises may be varied and, if desired, may be arranged to correlate closely with that in lectures, discussions, or readings. Some of the general exercises in Part I will be appreciated more fully if given after certain of those on animal types.

A minimum program for one semester may include the frog, histology, mitosis, maturation, embryology, classification, a field or museum exercise if practicable, and such animal forms as amoeba, paramecium, hydra, planaria, starfish, earthworm, and crayfish or insect or both. These may be studied intensively or other subjects added, according to the time, equipment, and facilities available.

A diversity of type faces is employed for ease of reference in the laboratory. Detailed instructions for students are printed in *italics* and some important terms in ***boldface italics;*** introductory remarks and the

lists of structural parts of animals are shown in smaller type. In each exercise the major topics are numbered (**1, 2,** etc.) and their subdivisions are lettered (***A, B,*** etc.) to facilitate the making of assignments. Specifications for drawings are placed at the end of each exercise since their inclusion in the text is often distracting. The tables for summarizing results of experiments may be copied out by students or the instructor may provide mimeographed copies to be filled out and turned in for grading.

Instructors who desire printed outline drawings for students to complete and label may order sets (Reed and Young, "Plates to Accompany Laboratory Studies in Zoology," McGraw-Hill Book Company, Inc.) from the publishers.

A pamphlet containing "Suggestions for Laboratory Instructors," designed to accompany this manual, is available upon request from the publishers. For each exercise it includes lists of suitable materials for use in class, by students or as demonstrations, together with methods for preparing such materials and formulas for the more commonly used solutions and media.

Some materials in this manual are derived in part from editions of the "Laboratory Instructions in General Zoology" used in the University of California since 1912. The author is obliged to Dr. Milton A. Miller for a critical review of the entire manuscript, to Dr. S. F. Light for comments on certain parts, to the Spencer Lens Company for the excellent illustrations of microscopes, to Emily Patterson Thompson for the line drawings, and to Nancy Mathews Webb for valuable aid in checking many details of procedure.

TRACY I. STORER.

DAVIS, CALIFORNIA,
August, 1944.

CONTENTS

STUDENT EQUIPMENT

Each student is to provide such of the following materials as directed by the instructor. *All personal equipment, where possible, should be marked with the owner's name.*

Student's name [illegible]
Laboratory section [illegible]
Laboratory hours 12-3
Seat No. 41 Room 208
Compound microscope No.....
Binocular microscope No.....
Locker No.....

.....Textbook
.....Laboratory manual for general zoology
.....Laboratory notebook
.....Drawing paper
.....Drawing pencil (3H, 4H)
.....Sandpaper pad for sharpening pencil
.....Eraser, soft rubber, beveled edge
.....Ruler, celluloid, millimeters-inches, about 6 inches long
.....Set of dissecting instruments, including
.....Scalpel
.....Scissors, straight, medium fine
.....Scissors, heavy
.....Forceps, straight, milled tips, about 5 inches long
.....Forceps, curved, milled tips, about 5 inches long
.....Dissecting needles, in handles
.....Probe
.....Pipettes (medicine droppers)
.....Case for instruments
.....Safety razor blade
.....Microscope slides, glass, 3 by 1 inches
.....Microscope coverglasses, No. 2, 7/8 inch (22 mm.), square or circular
.....Package of ordinary pins, or 20 glass-headed steel pins
.....Towel or piece of absorbent cloth
.....Magnifying glass
.....
.....
.....
.....

REFERENCE TABLES

All values on one horizontal line are equivalent (thus, 0.025 m. = 25.4 mm. = 1 in. = 0.0825 ft.). Standard abbreviations are shown in *italics*.

Linear Measure

Metric				United States and English			
Kilometers *km.*	Meters *m.*	Millimeters *mm.*	Microns *μ*	Inches *in.*	Feet *ft.*	Yards *yd.*	Miles *mi.*
1.	1000.	—	—	—	3280.8	1093.6	0.621
—	**1.**	1000.	—	39.37	3.281	1.093	—
—	0.001	**1.**	1000.	0.039	—	—	—
—	0.025	25.4	—	**1.**	0.0825	—	—
—	0.305	304.8	—	12.	**1.**	0.333	—
—	0.914	914.4	—	36.	3.	**1.**	—
1.61	1610.	—	—	—	5280.	1728.	**1.**

Fluid Measure

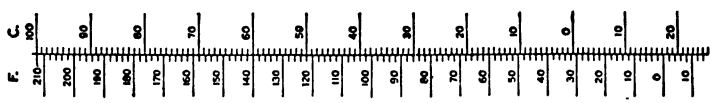
Metric		U. S. Standard			
Liters *l.*	Milliliters or cubic centimeters *ml.* or *cc.*	Fluid drams *fl. dr.*	Fluid ounces *fl. oz.*	Quarts *qt.*	Gallons *gal.*
1.	1000.	270.5	33.8	1.056	0.264
0.001	**1.**	0.271	0.034	—	—
0.004	3.69	**1.**(60 minims)	0.125	—	0.001
0.029	29.6	8.	**1.**	0.0625	0.008
0.946	946.3	256.	32.	**1.**(2 pints)	0.25
3.78	3785.3	1024.	128.	4.	**1.**(231 cu. in.)

Weight

Metric			Avoirdupois		
Kilograms *kg.*	Grams *g.*	Milligrams *mg.*	Grains *gr.*	Ounces *oz.*	Pounds *lb.*
1.	1000.	—	15,432.	35.27	2.204
0.001	**1.**	1000.	15.43	0.35	—
—	0.001	**1.**	0.015	—	—
—	0.065	—	**1.**	—	—
0.028	28.35	—	437.5	**1.***	0.062
0.453	453.6	—	7000.	16.	**1.**

* Apothecary or troy ounce = 31.103 grams = 480 grains.

Temperature Conversion Scale



To convert centigrade (°C.) to Fahrenheit (°F.): multiply °C. by 1.8 and add 32; thus, for 20°C., 20 × 1.8 + 32 = 68°F. To convert °F. to °C. subtract 32 and multiply by 0.55.

DEFINITIONS

For other terms see Glossary (and index) in Storer, "General Zoology."

Regions and Directions

Anterior. The forward-moving or head end in a bilaterally symmetrical animal, or toward that end; in front of. Opposite of *posterior.*

Posterior. The hinder part or toward the hinder (tail) end; away from the head. Opposite of *anterior.*

Dorsal. Toward or pertaining to the back or upper surface. Opposite of *ventral.*

Ventral. Toward or pertaining to the lower side or belly; away from the back. Opposite of *dorsal.*

Lateral. On or toward one side. Contrasted with *medial.*

Medial (median). On or near or toward the middle of the body. Contrasted with *lateral.*

Proximal. Nearer or toward the central part of the body. Opposite of *distal.*

Distal. Away from the place of attachment; toward the periphery or extremities of the body. Opposite of *proximal.*

Cephalic. Pertaining to or toward the head. Opposite of *caudal.*

Caudal. Pertaining to or toward the tail or posterior part of the body. Opposite of *cephalic.*

Axes

Main or longitudinal axis. A hypothetical line extending the length of the body from the anterior to the posterior end; or from the oral (mouth) surface to the aboral (opposite) surface in radial animals.

Dorso-ventral axis. From the back or dorsal surface to the under or ventral surface, and at right angles to the longitudinal axis.

Transverse axis. From side to side at right angles to the longitudinal and dorso-ventral axes.

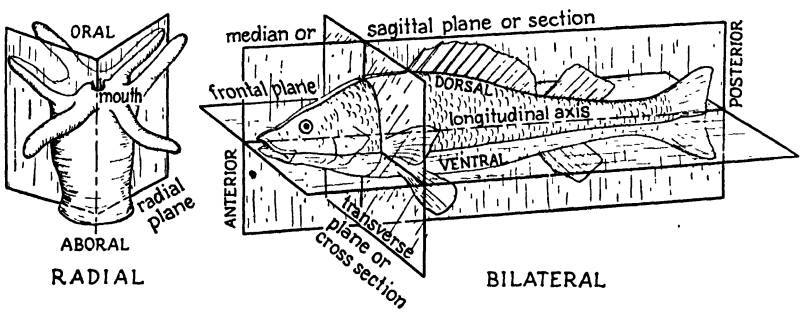


Fig. 1.—Types of symmetry and the axes and planes in animal bodies.

Planes

(along which the body may be divided or sectioned)

Median or **sagittal plane.** Divides the body into symmetrical right and left halves; includes the longitudinal axis and any dorso-ventral axis. Any plane parallel to the sagittal is a parasagittal plane.

Frontal plane. Any plane including a longitudinal and a transverse axis; hence parallel to the front of the body (man) or ventral surface (any bilateral animal); at right angles to sagittal plane.

Transverse plane. Any plane at right angles to a longitudinal axis; hence, at right angles to sagittal and frontal planes.

Symmetry

Asymmetrical. Any body or part that cannot be divided into two or more equivalent parts.

Radial symmetry. Having similar equivalent parts (antimeres) arranged around a common central axis, as in a sea anemone or starfish.

Bilateral symmetry. Having the body or a part that can be divided by one median (sagittal) plane into equivalent right and left halves, each a mirror image of the other.

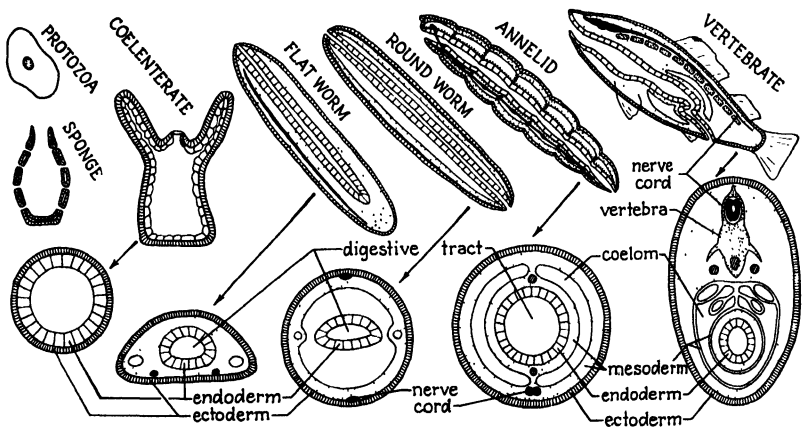


FIG. 2.—The body structure in various types of animals as seen in diagrammatic median and cross sections; the annelid and chordate show metamerism.

Metamerism. Having the body composed (externally, internally, or both) of a number of similar and homologous parts (somites, metameres), as seen in annelids, arthropods, and chordates.

INTRODUCTION

GENERAL INSTRUCTIONS FOR LABORATORY STUDY

1. Purpose. Personal experience provides the best basis for learning any subject. One may study alone but usually works more efficiently and progresses more rapidly when guided by a teacher with previous training and experience in the subject matter. Many facts, ideas, and conclusions may be learned from lectures or by reading, but this is "secondhand" information in the words of some speaker or writer. By contrast, laboratory study affords opportunity to obtain knowledge at firsthand from personal observation. The sciences deal with objects and their properties (text, pp. 3, 4), and laboratory study is important in the learning of any science. Such study constitutes training in scientific observation, which requires care and precision. In a zoological laboratory, students may learn directly of the structure, functions, and activities of animals.

2. General procedure. The laboratory instructor outlines the program to be followed, makes available the equipment and materials, demonstrates the methods to be used, gives individual students such special aid as they need, and subsequently examines them all on the knowledge that they have acquired. The oral and written instructions which are issued and those printed in the laboratory manual are designed to enable the students to work efficiently in respect to time and effort. The questions direct the students to make particular observations and to draw conclusions from the laboratory specimens, demonstration materials, and readings in the text or elsewhere. Each student, in fairness to his instructor and also to his fellow students, should be quiet, orderly, and attentive and should learn to work independently.

The instructor will designate which parts of the exercises in this manual are to be followed and will specify the drawings, tables, reports, or other written work to be prepared by the students. For each topic assigned, the student should *read, in preparation, before coming to the laboratory, the text account and the laboratory exercise assigned.* In the laboratory, after performing the work designated, it is well to read through the exercise once more to ensure that all details have been covered.

"Study nature, not books" was a phrase of Louis Agassiz, one of the great zoologists. The real source of zoological knowledge lies in the

animals themselves. The student therefore should investigate and seek to understand, as fully as possible, the materials provided in the laboratory. Any question of doubt is best settled by referring to the specimens.

3. Dissection. Study of animal structure commonly requires that a specimen be more or less taken apart, in an orderly fashion, to determine the structure and arrangement of its component parts. This process is termed "dissection." It requires neat and careful work. Often a laboratory dissection is planned to be progressive, by using one specimen to reveal various organs or systems in turn. The directions should be followed carefully to attain this result. Dissection consists of separating and exposing the parts of an animal body with as little damage to the specimen as possible. The forceps, probe, and needles are used more than the scalpel and scissors. The cutting and removal of parts should be done only as directed, and only after the parts are identified correctly and the purpose of the cutting is understood. Much dissection consists of loosening the connective tissues that bind organs and their parts together. Muscles should be separated parallel to their fibers, and blood vessels and nerves by working along rather than across them. The region being dissected should be kept moist at all times, wetting when needed, and all debris should be removed. Delicate or small dissections are best made and studied under water or other fluid which supports the parts and usually affords clearer vision of the area being dissected.

4. Drawings. The student's drawings are an important part of his record, comparable to the theme written in an English course or the report in a history class. The making of original drawings requires careful attention to details, hence is helpful in learning, and is also training in the preparation of scientific records. When graded by the instructor, drawings are rated on accuracy and completeness rather than on artistic merit, since the ability to draw varies widely among students. Copies of text illustrations as substitutes for original drawings are recognized readily by the instructor—and may be rated as cheating. Copying a fellow student's drawing may incorporate his errors and call for explanations from both students.

In making laboratory drawings, give attention to the following:

1. First observe and study the laboratory material as carefully as possible; then draw what is seen.

2. Draw directly from the specimens or slides, and complete drawings in the laboratory.

3. Arrange the separate figures on a sheet in a symmetrical pattern.

4. Make each figure large enough to show all details clearly. It should be simple and clear, mainly in outline, and necessarily somewhat diagrammatic.

5. Extensive repetition of repeated parts is unnecessary; instead, show a few with careful detail.

6. Use a sharp hard pencil (3H, 4H); avoid sketchy lines and soft or blunt pencils; do not ink or shade unless directed to do so.

7. First mark out lightly the extreme length and width (or diameter) to be occupied by a figure. If the specimen is bilaterally symmetrical, draw a faint longitudinal axis as a temporary guide in the placement of parts. Then outline the figure lightly. Estimate the proportionate size of some component parts in relation to the entire specimen, and mark these on the area outlined for the drawing. Draw the major features, fill in the details, and strengthen lines where necessary. Finally erase any unneeded marks.

8. Leave space, preferably at the right of the figure, for the labels. Label every part in each figure unless directed otherwise. Rule a fine line from each part horizontally to its label, and print or write each label neatly. Do not cross the lead lines.

9. For parts not seen in a specimen, consult any demonstration material and add the missing parts to the figure; otherwise note the part as "not seen" on the drawing.

10. Under each individual drawing give the figure number, title, and the approximate scale of reduction or enlargement as compared with the specimen, thus:

The Frog. Circulatory System, ventral view × 2

For drawings of microscopic subjects the lenses used may be noted: L.P. 5× (=low-power objective, 5× ocular).

11. On each sheet place your name and any other data indicated by the instructor (plate number, date, laboratory section, seat number, etc.).

5. Demonstration materials. In many laboratories additional specimens, microscopic preparations, and other objects may be displayed to supplement the materials used by individual students. Such demonstrations should be examined carefully as directed by the instructor to obtain fuller knowledge of the topic being studied.

6. Care of equipment. Property of considerable value is placed in the hands of students in a zoological laboratory and should be used with proper care. The same microscopes, slide collections, and other equipment often are used by different students in several successive laboratory sections. If any equipment assigned to a student is damaged or missing at the start of a laboratory period, this fact should be reported at once so that a charge may be placed against the previous user. Materials damaged while under use by a student also must be reported immediately. Crushing or breaking a prepared microscope slide may involve a charge of 50 cents to $2.50, depending on the actual cost of replacement.

MICROSCOPES

Small animals and objects are studied by use of some magnifying device that provides an enlarged image of the object. A simple biconvex hand lens serves for magnifications of a few diameters (Figs. 3 and 4). Doublet or triplet lenses (of two or three glass parts) will magnify up to 10 or 12 times; being corrected to overcome some optical defects of the simple lens, they provide better images. For greater magnifications a microscope (Gr. *mikros*, small + *skopos*, watcher) is necessary.

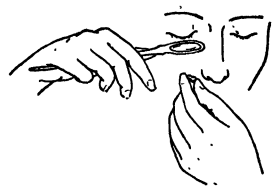


FIG. 3.—A hand lens or simple magnifier should be held close to the eye and the object brought up into focus. This method affords a maximum field of view.

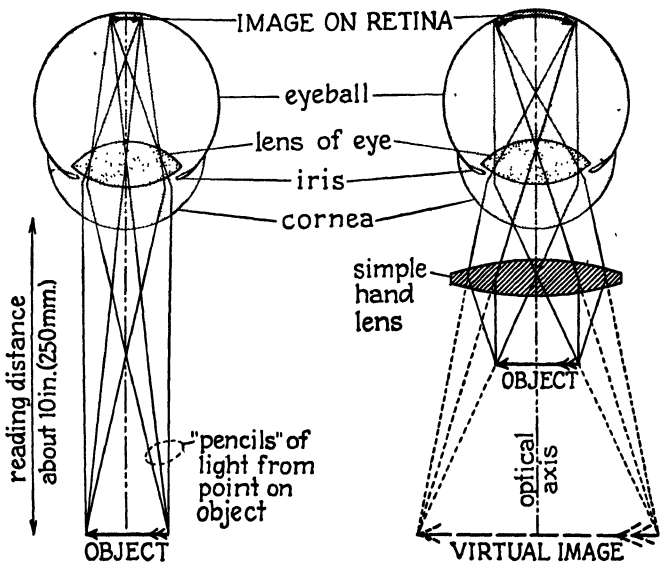


FIG. 4.—Formation of visual images. *Left.* The lens of the eye forms a real (inverted) image on the retina of the eye. "Pencils of light" are shown from only two points at the ends of the object (arrow), but such light pencils actually are reflected from every point on the object. *Right.* The simple hand lens "magnifies" the apparent size of an object by bending (refracting) the light rays to produce an enlarged image on the retina of the eye. The object is held in the principal focus of the magnifying lens. See p. 11 for explanation of virtual image (compare Fig. 3).

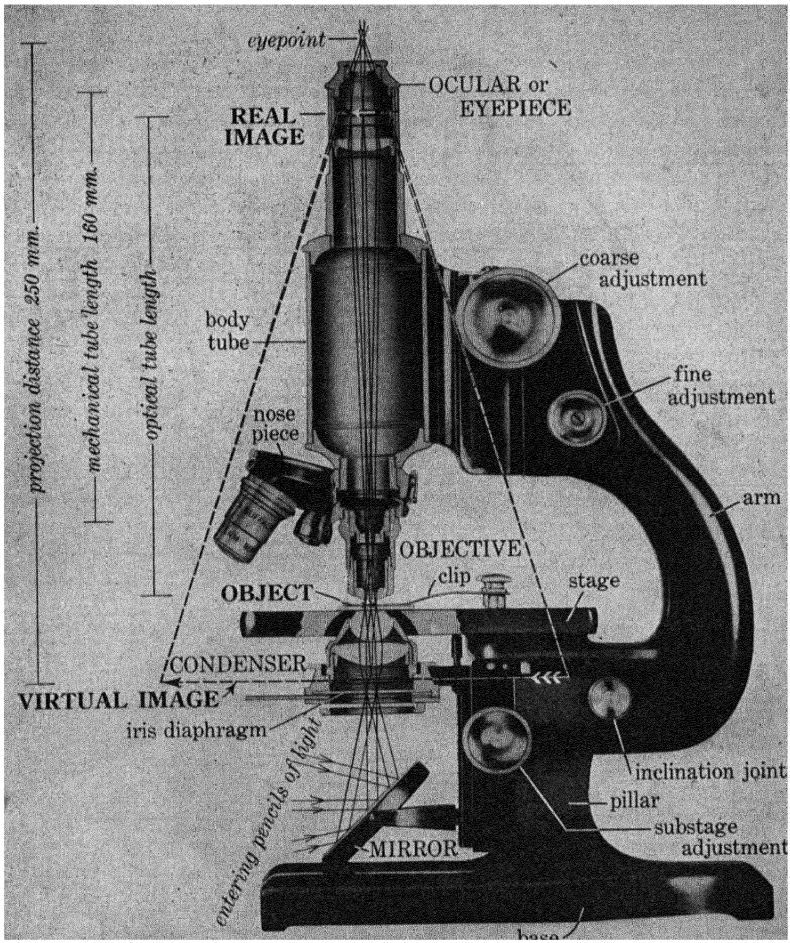


FIG. 5.—Compound microscope sectioned through the optical axis to show the optical parts and the paths of light involved in forming an image of the object (arrow). Direct paths of light rays are shown by solid lines. The apparent position and size of the virtual image is indicated by the large arrow (←——⟨) and the projection lines for this image by broken lines. (*Courtesy of Spencer Lens Co.*)

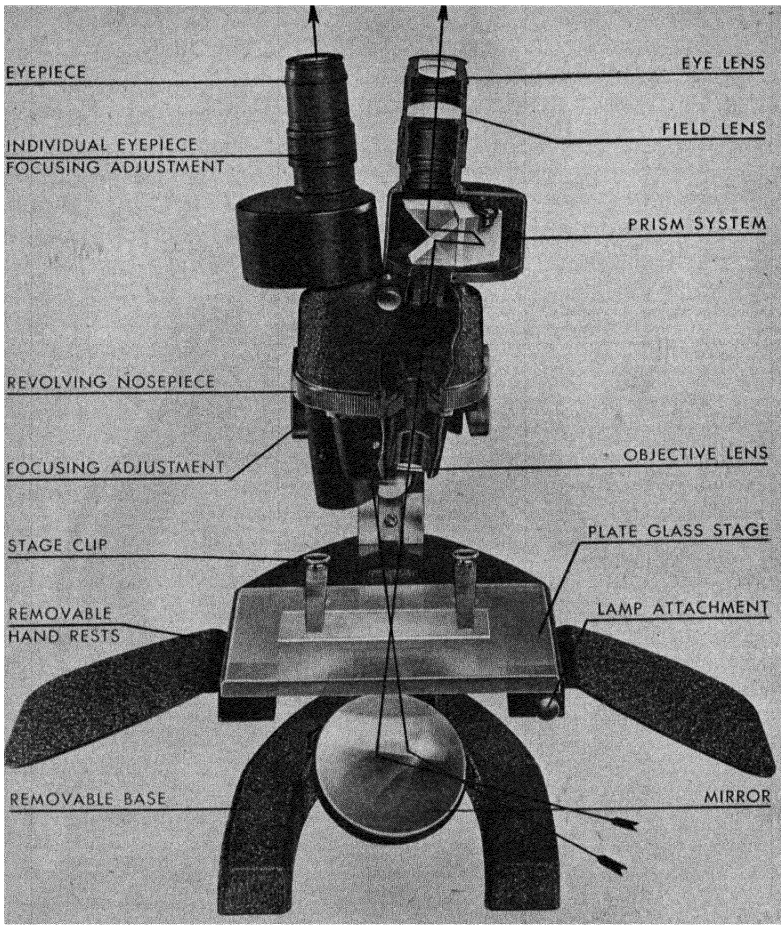


FIG. 6.—Binocular dissecting microscope sectioned through optical axis on one side to show optical parts and path of light rays (solid lines) that form an image for the eye from an object on the stage. (*Courtesy of Spencer Lens Co.*)

COMPOUND MICROSCOPE

1. Structure. A compound microscope (Fig. 5) consists of certain precise mechanical parts (chiefly of metal) to support and facilitate the use of the optical parts (mainly of glass) providing the magnified image.

A. MECHANICAL PARTS. These include: (1) the heavy ***base*** or foot; (2) the ***pillar,*** a firm support; (3) the ***inclination joint*** which permits of tilting the upper parts for convenience of the user; (4) the ***stage,*** a platform with two clips to hold the object or slide being studied; (5) the ***arm,*** a stout curved handle used in carrying the instrument; and (6) the ***body tube*** which bears the lenses. The lower lenses (objectives) commonly are borne on (7) a rotating ***nosepiece.*** Movement of the lenses to *focus* or obtain a sharp image of the object is accomplished by (8) the ***coarse adjustment*** connected to a small gear or pinion meshed with an upright toothed rack on the body tube. More delicate focusing, especially at high magnifications, is accomplished by (9) the ***fine adjustment*** or micrometer screw within the arm. At the top of the body tube in many microscopes there is (10) a ***draw tube*** which may be pulled upward to adjust the distance between the objective and ocular. The lenses of American microscopes are usually designed for a standard tube length of 160 mm. (170 mm. in Leitz microscopes).

B. OPTICAL PARTS. These consist of special types of glass, carefully ground and polished, and aligned on an optical axis. (1) The ***mirror,*** below the stage, serves to gather and direct light to illuminate the object. The concave mirror will gather more light than the flat (plane) mirror, but the latter provides more satisfactory lighting for high magnifications. Between the mirror and object is an ***iris diaphragm,*** of several blades (metal or fiber); these form a circular opening which may be enlarged or reduced to control the amount of light reaching the object. Many microscopes have also (2) a ***condenser*** (lens system) between the mirror and stage which serves further to concentrate light rays on the specimen. (3) The ***objective*** or object lens serves to form a real image of the object within the body tube. This may be seen by lowering an L-shaped slip of tissue paper into the tube after removing the ocular. (4) The ***ocular*** or eyepiece lens at the top of the body tube serves further to magnify this image. The lens of the ocular refracts (bends) the light rays passing from the real image to the retina of the eye in such a way as to produce the effect of a still larger ***virtual image*** (ghost image). The latter is imaginary (it cannot be projected on a surface); it produces on the eye the same effect as if an object the size of the virtual image were held at ordinary reading distance. An objective (Fig. 5) consists of two or more small lenses fixed in a rigid mount and an ocular of two larger lenses.

2. Care and cleaning. At the beginning of each laboratory period when the microscope is to be used, make sure that you have the proper instrument with all its parts in good condition. Clean the lenses of all oculars and objectives as necessary with lens paper; if dirty, breathe on the lens and then wipe gently. Never use anything else for cleaning the lenses. Report any shortage or damage to the instructor at once. Never attempt to take the microscope apart.

3. Coarse and fine adjustment. Use the coarse adjustment to rack the body tube about one inch above the stage. Note the direction of movement of the body tube. Do the same for the fine adjustment. Bear this in mind when focusing. The fine adjustment has a limited range; if resistance is felt, or if it no longer moves the objective, the limit has been reached; give the fine adjustment several turns in the opposite direction. Ask for assistance if difficulty is experienced.

On a student microscope with three objectives these commonly are termed low power (28 to 32 mm.), middle power (16 mm.), and high power (4 mm.); if only two are present, the 16-mm. objective is called the "low power."

4. Low-power objective. Place a test slide (Fig. 7) with letters, E or R, on the stage with the label side up; center one letter over the

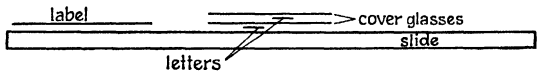


FIG. 7.—Sectional view of microscopic slide with two letters at different levels, for beginning exercise on compound microscope.

opening. If the microscope has a condenser, remove or swing it out to the side. Put the low-power (longer) ocular in the draw tube and rotate the nosepiece to bring the low-power objective (shortest) into the optical axis. Use the coarse adjustment to bring the tip of the objective about one inch above the slide. Turn the plane mirror so that light from the sky, or a white window shade, or a lamp is reflected up through the object and into the body tube. *Never use direct sunlight.*[1] Focus upward with the coarse adjustment until the object is clearly defined; then use the fine adjustment to obtain a sharp image. Recenter the letter in the field of view. What has the microscope done to the image of the object as to position? As to size?

5. Middle-power objective. Rotate the middle-power objective over the object and use the coarse adjustment to bring its tip about ¼ inch above the slide. Look into the ocular and focus up; then use the fine adjustment as before. Never focus down with the coarse adjustment

[1] The direct image of the sun if seen through a microscope may injure the eye by leaving a permanent afterimage.

while looking through the microscope. Why? Recenter the object by moving the slide, if necessary. Ask for help if needed.

Adjust the mirror for even illumination. Then turn the mirror to use the concave side. What is the effect? Turn the plane mirror up again, readjust, and put the condenser in place with the iris diaphragm wide open. (If there is an iris diaphragm above the condenser, never close it when the condenser is in place.) How does the amount of light received compare with that obtained without the condenser? Slowly close the diaphragm and observe carefully the effect on the illumination and the image.

For every object and at each magnification there is a certain light intensity at which a maximum of detail is seen; learn to recognize this condition and to adjust for it. With more light the details are lost, and with less light they disappear in the shadow. In either case the results are poorer and the eyestrain is greater. Most beginners use too much light. Keep both eyes open and relaxed when looking into a microscope; this reduces eyestrain. Proper use of a microscope, suitably adjusted, results in no more eyestrain than reading.

The magnification may be determined roughly by measuring the length of an object, then laying a small ruler on one side of the stage, and looking at the ruler with one eye while viewing the image of the object through the microscope with the other eye. (Use of stage and ocular micrometers with carefully ruled minute scales enables one to measure the magnification of microscopes and the size of microscopic objects carefully.)

6. High-power objective. Leaving the focus obtained with the middle-power objective unchanged, rotate the high-power objective (longest) above the object. If the two objectives are parfocal (instructor will explain), the high power will be in approximate focus. With the fine adjustment only, make a slight adjustment up or down as necessary. *Never use the coarse adjustment with high magnification.* The high-power objective is very close over the coverglass and can easily be forced into the latter, damaging both objective and slide. Therefore, be extremely careful in focusing downward. What is the effect of the change on the magnification? On the area of field covered?

7. Changing the ocular. Raise the objective about ½ inch above the slide, remove the low-power ocular, and insert the high-power ocular gently in the draw tube by a twisting motion. If it does not enter readily, ask for assistance. Dropping or forcing an ocular into place may push the objective downward and damage the latter or the slide. Now refocus with the middle-power objective or, watching from the side, lower the high-power objective until just above the coverglass, then look into the ocular and focus upward. What is the effect of the higher power ocular? In regular work use the lower power ocular whenever possible.

8. Light source. Use daylight whenever possible as it provides for better definition of microscopic structures and of the parts in stained preparations. If an image of the window or screen is seen at low or medium magnifications, use the concave mirror and lower the condenser slightly to get rid of the image.

When an artificial source of light is necessary, a blue-colored electric lamp bulb (or a blue "daylight" glass fitted below the microscope condenser) may be used to render the artificial light more like daylight.

9. Practice. Focus with various combinations of objectives and oculars; learn how to locate an object or part of it readily; and study the adjustment necessary in illumination. Demonstrate to the instructor your ability to change from one power to another and to adjust the lighting.

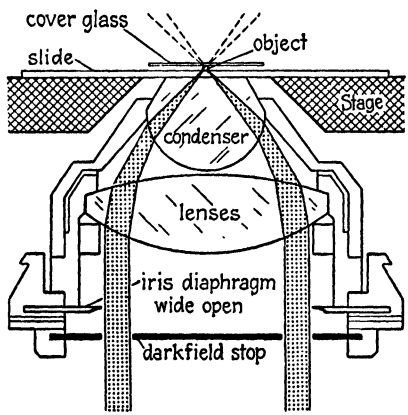


FIG. 8.—Section through the condenser and stage of a compound microscope to show the principle of darkfield illumination. The darkfield stop permits only a hollow or cone of light (dotted) to pass through the outer parts of the condenser lens and illuminate the sides of the object (O) by reflected light.

Constant use of the fine adjustment is necessary at medium and high magnifications because the depth of field that is in sharp focus becomes less and less with increasing magnification. Such use aids the eye in obtaining a sharply focused image and assists in interpreting correctly the images at different levels within a preparation. *An optical section consists of the field that is in sharp focus at any plane below the surface of a more or less transparent specimen when seen through the microscope.* Several optical sections may be distinguished even in a very thin microscopic preparation.

With wet preparations (water mounts, etc.) take care to have the stage level and to keep water or other liquids from getting onto the objectives or the substage parts.

Place a few fibers of filter paper or cotton, fine threads of cloth, or a hair on a slide, add a drop of water, and try to include some air bubbles when laying on the coverglass. Some fine particles of dirt, carbon, or carmine may also be included. Examine at various magnifications to learn the appearance of these objects under the microscope. The instructor may provide water containing microscopic animals or plants or other materials for examination.

10. Darkfield illumination. The compound microscope with a condenser usually has a slot at the bottom of the condenser mount into which a metal darkfield stop (Fig. 8) may be inserted. This permits only a hollow cone of light to pass through the outer parts of the condenser lenses and illuminates the object by reflected light, leaving the surrounding field dark. This is termed "darkfield illumination." A strong light source is needed (such as a 25- to 40-watt electric bulb close by), and the iris diaphragm must be wide open. Some details of microscopic animals are seen more readily under this illumination (such as the action of cilia and flagella in living material), although the definition is rather poor except in a microscope specially equipped for darkfield use.

BINOCULAR DISSECTING MICROSCOPE

This instrument (Fig. 6) has paired objectives and oculars to provide binocular (two-eyed) vision, whereby the relations of parts at different levels in a specimen may be determined. It contains a series of prisms that produce an "erect" image (not inverted as in a compound microscope). The magnification is about 5 to 50 times, depending on the objectives and oculars supplied, and there is only a coarse adjustment for focusing. Means are provided to adjust the two oculars for the distance between the eyes (interpupillary distance) of the user; also the tube supporting one ocular may be focused separately to compensate for differences between the eyes of the user. This microscope is particularly useful for examining small specimens or parts and for performing delicate dissections.

Place a slide bearing a mounted letter or some other small object on the stage. Close the eye over the adjustable ocular, and while looking through the other (fixed) ocular bring the object into focus by use of the focusing knob. Then open the eye over the adjustable ocular and move the latter up or down until the images for both eyes are sharp. Finally set the distance between the two oculars until the two images are seen as one.

What is the approximate magnification?

If the dissecting microscope has additional paired objectives (on a nosepiece) or another pair of oculars, place these in position and learn their effect on magnification and on the size of field covered.

MAGNIFYING POWERS OF MICROSCOPES

The "magnification" by a lens or microscope means the number of times that the image of an object is enlarged in length (linear magnification) as compared with the apparent size of the same object when held at the ordinary reading distance of about 10 inches (250 mm.). The total magnification in either a compound or a binocular microscope is the product of the separate magnifying powers of the objective and ocular (figures in *italics* in table).

Spencer, Bausch & Lomb, etc., microscopes						E. Leitz microscopes				
Objectives			Oculars (eyepieces)			Objectives			Oculars	
Focal length		Magnification	5 ×	6 ×	10 ×	No.	Mm.	Magnification	6 ×	10 ×
Mm.	Inches									
32	1¼	× 5	*25*	*30*	*50*	1*b*	32	× 4.3	*26*	*43*
16	⅔	× 10	*50*	*60*	*100*	3	16.2	× 10.3	*62*	*103*
8	⅓	× 20	*100*	*120*	*200*	4	10	× 19	*114*	*190*
4	1/6	× 43	*215*	*258*	*430*	6*a*	4.2	× 44	*264*	*440*
1.8*	1/12	× 93	*465*	*558*	*950*	1/12*	2	× 92	*552*	*920*

*Requires cedarwood immersion oil between objective and coverglass; the latter must be of No. 1 thickness (0.12 to 0.18 mm.).

MICROSCOPIC PREPARATIONS

Microscopic objects are those too small to be seen or studied readily by the unaided eye, which usually cannot discriminate (resolve) items less than 0.15 mm. apart. Some objects may be examined under the microscope as they exist and others in temporary mounts in water or other fluids, but many must be stained to differentiate their component parts. They may be prepared as "whole mounts" of entire specimens, or as microscopic sections, in either case mounted on glass microscopic slides (usually 1 by 3 inches) and sealed under thin coverglasses (see also Exercise 1).

For permanent whole mounts, the specimen is killed and preserved in an appropriate "fixing fluid," then washed, stained, dehydrated (in alcohols of progressively higher concentration), cleared, and mounted in a transparent medium (commonly Canada balsam) between a slide and coverglass.

Entire animals and pieces of tissue too thick or too opaque to observe and study in their natural condition must be cut into thin slices or sections. After killing, fixing, washing, dehydrating, and clearing, the

materials (small animals or parts of larger ones) are immersed in melted paraffin until all portions of the specimen are infiltrated by the latter. Then each is cast in a small block of paraffin. The embedded specimen is then cut into extremely thin sections (commonly 10 *microns* [μ] = 0.01 mm. = 1/2,540 inch thick) on a precision machine known as a microtome (Gr. *micros*, small + *toma*, to cut). These sections are fastened to

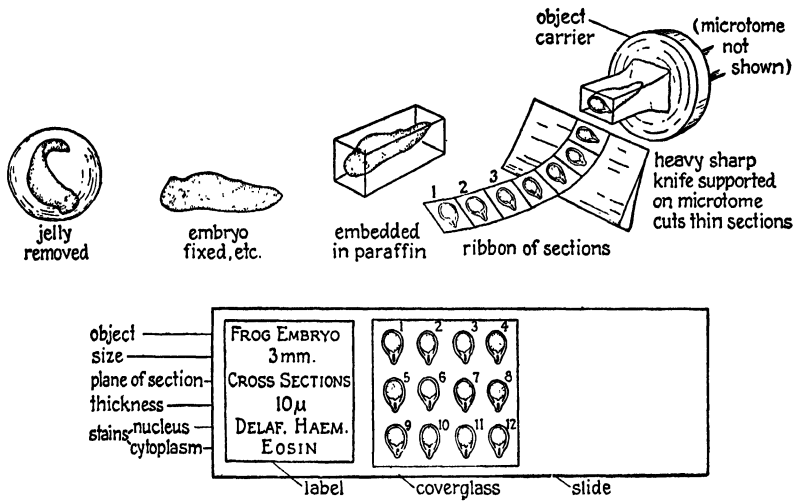


FIG. 9.—Principal steps in preparing microscopic sections for study. Serial sections usually are arranged in the numerical sequence indicated for convenience.

microscope slides with an adhesive, and the paraffin that served for support during the cutting is dissolved away. The sections are stained as necessary, cleared, and sealed in a thin film of Canada balsam under a coverglass (0.25 to 0.12 mm. thick). The principal stages in this process of microscopical technique may be illustrated with a frog embryo cut into serial cross sections (Fig. 9).

PART I. GENERAL ANIMAL BIOLOGY

EXERCISE 1. CELLS, TISSUES, AND ORGANS

(Storer, "General Zoology," pp. 45–47, 51–59)

The bodies of all organisms—plants and animals—are composed of the living substance known as ***protoplasm.*** This is organized into microscopic units known as ***cells.*** A cell consists of a central kernel or nucleus surrounded by cytoplasm and is enclosed in a cell membrane. The cell is the basis of both structure and function in all living things. In the lowest forms of life (unicellular plants and PROTOZOA) the individual consists of a single cell, whereas in all other organisms the body consists of many cells of various kinds.

In a multicellular animal, the cells of each kind are aggregated to form subdivisions or layers known as ***tissues;*** and tissues of several kinds are grouped to form ***organs.*** The stomach, for example, is an organ composed of several kinds of tissues, each made up of particular kinds of cells; and each tissue performs some definite function in the general activities of the stomach. The study of tissues is known as ***histology*** or ***microscopic anatomy*** because animal cells are so small that they must be examined under high magnification with the compound microscope.

Certain tissues can be examined merely by separating the component cells. Some are carefully ***teased*** apart with needles and placed in normal salt solution. This contains a concentration of ordinary salt (NaCl) equivalent to that in the living animal and serves to keep the material alive and in natural condition. Other tissues are ***dissociated*** or ***macerated*** by special fluids that dissolve the cementing substances holding the cells together. Most histological study, however, requires that the tissues be removed in small pieces, ***fixed, sectioned*** into thin slices, ***mounted*** on microscopic slides, and ***stained*** to differentiate the cell components. A wide variety of chemical fixing agents and stains is used for different tissues. "Prepared slides" represent a considerable investment of materials and much skilled workmanship. They must be used with care and be protected against crushing or breakage. A single slide or section often contains more than one type of tissue or cell so that it is necessary to search for the portions wanted in any particular study.

This exercise will afford (1) a practical introduction to the use of the microscope, (2) a study of examples of the principal kinds of cells and tissues, and (3) an examination of some representative organs.

CELLS

1. Cellular structure. *Examine a stained microscopic section of some part of an animal* (liver, etc.) *under medium magnification and notice that it is composed of a great many small cells. Then, under high magnification, identify the principal differentiated parts of the protoplasm comprising one cell:*

Cell membrane (surrounds cell)
Cytoplasm (material between cell membrane and nuclear membrane)

Nucleus (central, darker or more heavily stained)
Nuclear membrane (surrounds nucleus)
Chromatin (densely staining parts within nucleus)

Draw Fig. 1

2. Epithelial cells. *Gently scrape the inside of your cheek with a toothpick; mount the scrapings in water on a slide, and lay on a coverglass. Place one drop of acetocarmine stain at the edge of the coverglass and allow it to diffuse into the preparation. Under medium and high magnification, find the loose epithelial cells from the lining of the mouth cavity; identify the cell parts named in Par.* 1 *so far as possible.*

Draw Fig. 2

3. Frog egg. The animal egg is a single cell, but differs from other cells in being much larger and in being set free when it becomes mature. *From a fragment of fresh frog ovary, gently tease free one or more eggs* (ova); *mount on a glass microscope slide in* 0.7 *per cent salt solution and examine under medium magnification. In addition to the parts named in Par.* 1, *find granules of pigment and yolk.* The chromatin in an unstained egg nucleus appears as yellowish refractive granules.

TISSUES

The cells comprising the body of a multicellular animal are organized in groups or layers, the ***tissues,*** which comprise five major types: (1) epithelial or covering, (2) connective and supportive, (3) vascular or circulatory, (4) contractile or muscular, and (5) nervous. The germ cells that serve in reproduction to continue the species are considered in Exercises 12 and 13.

1. Epithelial tissues. These are arranged in thin layers, termed ***epithelium,*** that cover various surfaces, both external and internal, in the body.

A. SQUAMOUS EPITHELIUM. The thin outer layer shed from the skin of frogs in an aquarium is an example of squamous epithelium. *Study a prepared slide, or else obtain a small piece of fresh material, spread in a drop of water on a slide, and lay on a coverglass; examine under medium magnification with weak illumination.* The thin flat polygonal cells are arranged somewhat like the small tiles in a mosaic or pavement. *Under high magnification search within the cells for pigment granules and the nuclei* (staining with acetocarmine will render the nuclei more visible). *Focus up and down to determine the number of cell layers present.*

From human skin the dry outer layers of cornified epithelium slough off continually in minute dry pieces (but thickened portions, as over a dried blister, come off in larger pieces).

Draw Fig. 3

B. COLUMNAR EPITHELIUM. (1) If a short length of small intestine from a freshly killed frog is immersed in 30 per cent alcohol for 12 to 24 hours, the cells lining the interior cavity or lumen will become loosened or dissociated. *With forceps, grasp a short fragment so prepared and shake off some of the cells into alcohol on a slide, add a coverglass, stain if necessary with acetocarmine, and examine;* or (2) *study these cells in a stained cross section of stomach or intestine.* The erect tall columnar cells are closely placed. Besides the nucleus in each, some may show minute secretory granules near the inner or exposed end.

Draw Fig. 4

C. CILIATED EPITHELIUM. (1) *Gently scrape the roof of the mouth in a freshly killed frog, mount the scrapings in* 0.7 *per cent salt solution on a slide under a coverglass, and examine under reduced light.* The shimmering movement is produced by the many fine hair-like ***cilia*** on the ends of the cells; as the movement slows, the cilia may be seen. Under darkfield illumination cilia often show clearly.

(2) A stained section of the windpipe or trachea of a mammal will show columnar ciliated cells lining the interior. These cells show a false or pseudostratification into layers.

What is the stroke of an individual cilium? What does ciliary action accomplish? In what organs of the frog do cilia occur? In what organs in man?

Draw Fig. 5

D. CUBOIDAL EPITHELIUM. *Study a stained section of kidney.* The many irregular cavities are portions of minute tubules lined with somewhat cubical cells; these form an epithelium that is also glandular or secreting. Similar epithelium is found in parts of the salivary glands in the mouth, and in other glands.

How do these cells differ from the preceding types?

Draw Fig. 6

2. Connective and supportive tissues. The parts of an animal body are bound together and supported by connective tissues, cartilage, and bone. In such tissues the few cells are scattered amid much intercellular material that the cells have secreted.

A. CONNECTIVE TISSUES. *Study a stained cross section of stomach or intestine and between the larger obvious "layers" find, under high magnification, areas containing many long fine wavy connective-tissue fibers.* These are thought to lie in an invisible gelatinous matrix. Some nuclei are narrowly surrounded by cytoplasm, and some cells have irregular strands or processes extending among the fibers.

Draw Fig. 7

B. CARTILAGE. *In a special stained slide of cartilage or in a section of a larval salamander, find areas of the clear cartilage matrix* (stained bluish or pinkish) *that contain many small groups of cartilage cells;* the latter lie in spaces (lacunae) within the matrix.

Draw Fig. 8

C. BONE. Living bone consists of an organic matrix (collagen) and deposits of mineral materials secreted by special bone-forming cells, the ***osteoblasts.*** Sections of bone may be prepared in two ways: (1) by grinding pieces of dry bone until thin, or (2) by dissolving away the hard parts with acid (decalcification) so that the remaining portions can be cut as microscopic sections. *In a complete transverse section of some long bone from a limb, identify:*

Periosteum (external thin covering of connective tissue that contains osteoblasts)
Periosteal lamellae (thin parallel layers of bone substance beneath periosteum)
Concentric lamellae (groups of small concentric layers comprising the bone substance medial to the periosteal lamellae)
Haversian canals (in center of each group of concentric lamellae; serve as paths of blood vessels within the bone)
Lacunae (microscopic spaces between lamellae; in life occupied by osteoblasts, which have many slender processes extending into fine canaliculi in the lamellae)
Marrow cavity (space in center of bone)

Draw Fig. 9

3. Vascular tissues. The blood consists of a fluid, the ***plasma,*** containing free blood cells or ***corpuscles*** of two kinds: many red blood cells (erythrocytes) and fewer white blood cells (leucocytes). The red blood cells of mammals are nonnucleated after their growth and entry into the blood stream, but all other types are nucleated.

A. FROG BLOOD. *Obtain a drop of fresh frog blood on a clean slide, add a drop of* 0.7 *per cent salt solution, lay on a coverglass, and examine under medium and high magnification* (text, Fig. 2·12); *distinguish:*

Erythrocyte (elliptical, colored red or yellow by hemoglobin, and with central oval nucleus)
Leucocyte (varied in shape, about one-third size of erythrocyte, cytoplasm clear or granular; often shows amoeboid movement; reduce illumination to see)

Draw Fig. 10

B. HUMAN BLOOD. *Make a similar preparation of human blood and examine.* The erythrocytes are much smaller (7 to 7.5 μ) than in the frog, and are circular, biconcave, and nonnucleated. The leucocytes resemble those of the frog (text, Table 4·3, Fig. 3·10).

C. *Examine also, if available, stained smears of both types of blood.*

4. Muscular tissues. Microscopically, the muscles of vertebrates are of three types: (*a*) smooth or nonstriated, the involuntary muscle of

internal organs; (*b*) skeletal or striated, the voluntary muscles attached to the skeleton that produce bodily movements; and (*c*) cardiac, present only in the heart. Individual muscle cells are slender, long, and contain minute lengthwise ***fibrillae.***

A. SMOOTH MUSCLE. (1) *Tease out a piece of macerated frog intestine in fluid on a slide, add a coverglass, and under high magnification find the long muscle cells; each is tapered at both ends and has a central oval nucleus.*

(2) *Study a stained cross section of stomach or intestine; in the outer part is a thick layer of circular muscles* (fibers seen in longitudinal section) *and outside of these a thinner layer having small bundles of longitudinal muscles* (seen in cross section). *Determine the shape and structure of the cells.*

Draw Fig. 11

B. STRIATED MUSCLE. Each fiber is a slender cylindrical cell (1 to 40 mm. long) enclosed in a delicate membrane, the ***sarcolemma,*** and each contains many nuclei. The fiber is made up of many lengthwise fine fibrillae, which have distinct ***cross striations,*** alternately dark and light.

(1) *Place a small piece of fresh frog muscle in* 0.7 *per cent salt solution on a slide; with needles carefully tease apart the fibers, lay on a coverglass, and study under high magnification.* The sarcolemma shows best where fibers are crushed or torn. *A drop of acetic acid will make the nuclei more distinct.*

(2) *Examine a stained section of striated muscle* (tongue of mammal or body and tail of larval salamander are favorable materials). *Find the fibers, nuclei, fibrillae, and striations. Notice how connective tissues join and bind the muscles.*

Draw Fig. 12

5. Nervous tissues. Nerve cells or ***neurons*** of various types make up the nervous system (brain, nerve cord, nerves). The cell body of a neuron contains the nucleus, and extending from the cell body are one, two, or more slender cell processes, short or long (text, Fig. 3·12). A ***nerve*** consists of fibers, which are the processes from many neurons; some are centimeters in length. The brain and nerve cord are composed of great numbers of neurons and their processes.

A. NEURONS. *Examine a smear made from fresh nerve cord, fixed, and stained; find several types of neurons. In each distinguish the cell body, nucleus, and cell processes.*

Draw Fig. 13

B. NERVE. *Study a stained cross section of a nerve.* This consists of the processes (nerve fibers) of many neurons, bound together and surrounded by connective tissue. Each comprises a central darkly staining

axis cylinder (the fiber) surrounded by a clear ***medullary sheath*** of fatty nature; around the latter is a delicate membrane or ***neurilemma.***

Draw Fig. 14

C. *Examine any other preparations of nervous tissues that may be demonstrated.*

ORGANS

An organ is a tissue complex, being composed of two or more types of tissues associated to perform some general bodily function. Stained microscopic sections of organs will show how the various cell types and tissues are combined.

1. Frog skin. The skin of a frog serves several functions, including (*a*) physical protection of the body; (*b*) biological protection against the invasion of foreign organisms; (*c*) respiration, the exchange of oxygen and carbon dioxide; and (*d*) production of useful secretions to keep the skin moist for respiration and slippery to avoid capture by enemies. The skin consists of two major parts, the outer ***epidermis,*** which is stratified, and the ***dermis*** (corium) beneath (text, Fig. 2·6).

Examine a stained section of the skin at medium and high magnifications; identify:

EPIDERMIS

Cornified layer (outermost, very thin, often partly broken)
Intermediate layers
Germinative layer or Malpighian layer (at base of epidermis; of columnar cells with large nuclei)
Pigment granules (minute, blackish)
Epidermal glands (multicellular; their bases extend into dermis)

DERMIS

Outer spongy part (contains blackish chromatophores, the bases of glands, blood capillaries, and scattered connective tissue fibers)
Thick middle part (alternately crossed layers of connective tissue fibers; nuclei few)
Inner compact part (over lymph spaces beneath skin)

Draw Fig. 15

2. Wall of stomach or intestine. *Examine a stained cross section of the stomach or intestine of a frog or salamander and under low magnification distinguish the four principal layers from the interior cavity* (lumen) *outward* (text, Fig. 4·11); *then study the cells and tissues of each under high magnification; identify:*

Mucosa (innermost; mainly of columnar epithelial cells, but also scattered oval goblet cells with clear contents; the mucosa produces a sticky mucus, for which it is named)
Submucosa (outside mucosa; contains bases of multicellular glands opening into lumen, also blood vessels, nerves, and connective tissue fibers)

Muscularis (smooth muscle cells; of 2 parts; thick inner circular layer, the cells cut lengthwise; and thinner outer longitudinal layer, somewhat in bundles, the cells cut transversely)

Serosa (outermost; very thin single layer of squamous cells, in cross section; produces a watery or serous secretion; comprises the peritoneum covering this and other organs within body cavity)

Between and within the layers, especially the submucosa, are various connective tissue fibers and cells, binding the parts together. Small blood vessels are recognized by their thin walls (of endothelium) and their content of blood cells; the nerves are small bundles of solid fibers.

Draw Fig. 16

DRAWINGS

In each figure draw carefully only a few cells, but make them of sufficient size to show clearly the details of structure in each and label.

Fig. 1. Animal cell (25 mm. in diameter); label parts listed in Par. 1 (cells).

Fig. 2. Epithelial cells of mouth cavity.

Fig. 3. Squamous epithelium from frog skin, in surface view.

Fig. 4. Columnar epithelium.

Fig. 5. Columnar ciliated epithelium.

Fig. 6. Cuboidal epithelium.

Fig. 7. Connective tissue fibers and cells.

Fig. 8. Cartilage matrix and cells.

Fig. 9. Section of bone; show a few lamellae.

Fig. 10. Blood corpuscles of frog (red corpuscle about 25 mm. across, others in proportion). Show red corpuscle in both surface and edge view if possible; label cell membrane and nucleus.

Fig. 11. Smooth muscle.

Fig. 12. Striated muscle.

Fig. 13. Types of nerve cells; show several.

Fig. 14. Cross section of nerve.

Fig. 15. Section of frog skin (50 mm. high); outline and label the parts named in Par. 1 (organs) and show a few cells in each.

Fig. 16. Cross section of stomach or intestine; show only a narrow radial sector from the interior (lumen) to the outside; outline the layers, show a few cells in each, and label.

EXERCISE 2. THE FROG: EXTERNAL FEATURES AND MOUTH CAVITY

(Storer, "General Zoology," pp. 15–21)

Frogs live in water and in moist places on land and have various special adaptations in structure and function that enable them to live successfully in both of these environments. The leopard frog (*Rana pipiens*) and the bullfrog (*Rana catesbeiana*) serve well for introductory study because they are easy to obtain and large enough for the beginning student to dissect readily, and because the anatomy of a frog resembles that of the human body in many features. Live specimens are used for the study of behavior and for many physiological experiments.

Frogs are collected most easily after dark in marshy places with the aid of a spotlight. They may be kept alive for some days in a moist enclosure at room temperatures, and longer at 50°F. or lower. Specimens for dissection are killed with an anesthetic such as ether. They may be dissected immediately but commonly are preserved against decay in formalin (5 to 10 per cent solution). Preserved specimens should be washed well before being handled and at times during dissection, because the formalin tends to harden human skin and its vapors are irritating to the mucous membranes of the eyes and nose. Specimens should be kept moist while being studied and put away at the end of each laboratory period as directed by the instructor.

Dissection of the frog comprises a series of exercises in which all parts of a specimen will be used in turn. Instructions must be followed carefully, and no part should be cut or discarded until directions are given to do so. When examining any small part under a hand lens or binocular microscope, disturbing surface reflections will be avoided if it is placed under water.

1. Anatomical terms. *Learn the meaning and application of the following terms in respect to the frog and also to the human body.* These are used in describing the parts of animals and in directions for study and dissection (see p. 3 and Fig. 1).

Anterior and posterior
Dorsal and ventral
Median and lateral
Proximal and distal
Right and left (as applied to a specimen)

2. External features. *Using a fresh or preserved frog, identify:*

Head (to behind eardrum)
Trunk (remainder of body)
Snout (region anterior to eyes)
Mouth
Anus (opens dorsally at end of body)
Fore limb (upper arm, forearm, hand, wrist, 4 digits or fingers)
Hind limb (thigh, shank or lower leg, ankle, foot, 5 digits or toes, webs)
Nostrils or external nares (2; small, on snout)
Eyes, each with
  upper eyelid (fleshy)
  lower eyelid (narrow)
  nictitating membrane (large thin eyelid, inside other two)
  cornea (transparent surface of eye)
  iris (colored)
  pupil (opening in iris)
Tympanic membranes or eardrums (behind eyes)

The innermost digit of each hand is enlarged in males.

Draw Fig. 1

Visualize the longitudinal axis through the head and trunk. What plane would divide the frog (or man) into right and left halves? What is bilateral symmetry? Is there a distinct neck? What produces the hump in the back (examine a mounted skeleton to determine)?

3. Mouth cavity. *Open the frog's mouth widely by bending the lower jaw far back; cut the muscle at the corner of the mouth if so directed. Wash out any mucus present. Find the following:*

ROOF OF MOUTH CAVITY

Maxillary teeth (on upper jaw, many, minute)
Vomerine teeth (2 small patches, between internal nares)
Internal nares (2 anterior openings; connect to external nares)
Openings of Eustachian tubes (2; at corners of mouth; each connects to cavity of middle ear under eardrum)

FLOOR OF MOUTH CAVITY

Tongue
Openings to vocal sacs (2; lateral; present only in males)
Hyoid cartilage (embedded below tongue)
Pharynx (posterior part of cavity, behind tongue)
Glottis (lengthwise slit in ventral wall of pharynx)
Esophagus (posterior to pharynx)

On the upper and lower jaws, find the "interlocking" structures (grooves and ridges, tubercle and depression) *that make for tight closure of the mouth.* Why is such closure necessary?

Draw Fig. 2

Are lips present? Do the teeth serve for biting, chewing, or holding prey (food)? How is the tongue attached? How is it used (text, Fig. 29·5)? Of what service is mucus in the mouth? Of what practical advantage is the large mouth?

4. Skin or integument (see also Exercise 1). ***A.*** *Under a lens or binocular microscope find the minute pores from multicellular glands in the skin.* These glands produce mucus that makes the skin slippery and aids in keeping it moist. In a live frog what is the texture of the skin? Is it firmly or loosely attached? What are the advantages and disadvantages of such a skin to the frog? How does the skin coloration differ on the upper and under surfaces of the body? Do these colors afford the frog any advantage in its natural environments? What produces the green color?

B. *Cut off some skin from the trunk and examine the inner surface under low magnification; note the many blood vessels.* What is their function? Pigment cells are present along many of the vessels.

5. Behavior. *Observe a frog when resting and also when leaping.* What is the normal posture? How do the limbs act when leaping? Is the resting position of advantage when danger threatens? Of what

advantage is the moist slippery skin for safety? *Watch a frog in deep water to see its actions when swimming, diving, and floating.*

DRAWINGS

Fig. 1. Frog (70 mm. long); in lateral view; label external features.

Fig. 2. Mouth cavity of frog (50 mm. long), slightly from one side; label.

EXERCISE 3. THE FROG: SKELETON

(Storer, ' General Zoology," pp. 21–26; 62–70)

The skeleton is a jointed internal framework that supports the soft parts of the body, protects the more vital organs, and provides attachments for the muscles used in movement and locomotion. In a frog larva (tadpole) it is entirely of cartilage, but in the adult frog it is chiefly of bone with cartilage on the ends of the limb bones, in parts of the skull, and in the limb girdles. The skull, vertebral column (backbone), and sternum (breast bone) comprise the median or axial skeleton; the paired fore and hind limbs together with the supporting shoulder and hip girdles form the appendicular skeleton (text, Table 4·1). The frog has no ribs.

Skeletons for study are prepared by "stewing" a freshly killed frog in mild alkali (washing powder, etc.) to soften the flesh which then is removed by scraping and brushing. Care is needed to preserve the soft cartilages and to prevent some parts from being separated (disarticulated).

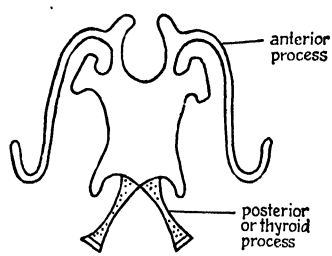


FIG. 10.—Outline of the hyoid cartilage of a frog in ventral view; enlarged.

1. The skull. ***A.*** The conspicuous subdivisions are (1) the slender median cranium or brain box housing the brain; (2) the paired jaws, upper and lower; and (3) the paired capsules for organs of special sense (*a*) nasal or olfactory, (*b*) ear or auditory, and (*c*) eye or optic (this consists of sclerotic cartilages in each eye). There is a spacious orbit for each eye. Embedded beneath the tongue is the thin flat ***hyoid cartilage*** (Fig. 10) with processes. This is often lacking in a cleaned skeleton, as is the slender columella bone of each middle ear.

B. *Identify the bones* (all paired except parasphenoid) *beginning at the anterior end:*

Dorsal Side	Upper Jaw	Ventral Side
Nasal* (triangular)	Premaxilla* (with teeth)	Vomer* (with teeth)
Frontoparietal* (slender)	Maxilla* (with teeth)	Sphenethmoid
Exoccipital (short, with occipital condyle)	Quadratojugal*	Pterygoid
	Lower Jaw	Parasphenoid* (single)
Pterygoid	Mentomeckelian	Exoccipital
Squamosal*	Dentary*	Palatine
Prootic (squarish)	Angulare*	Prootic

(Dermal bones, formed during development by ossifications in the deeper layer or dermis of the skin, are marked *; all others are cartilage bones formed by replacement of cartilage.)

The large posterior opening between the occipital condyles is the ***foramen magnum*** through which the brain and spinal cord connect. There are paired openings or ***foramina*** for exit of cranial nerves in the sides of the cranium.

Draw Fig. 1

2. Vertebral (spinal) column. There are 9 separate vertebrae and a narrow blade-like urostyle. *In any one vertebra* (4th, etc.) *identify:*

Centrum (solid, cylindrical, ventral)
Neural arch (dorsal to centrum, encloses nerve cord)
Neural spine (projects dorsally from arch; for muscle attachments)
Transverse processes (2; lateral to centrum and neural arch; for muscles)
Zygapophyses (2 pairs; small projections at both ends of neural arch, by which the vertebra articulates on those adjacent)

The first vertebra (atlas) lacks transverse processes and is modified anteriorly to receive the two occipital condyles on the skull by which the latter articulates with the vertebral column. The 9th or sacral vertebra has enlarged transverse processes to which the pelvic (hip) girdle attaches. Between adjacent neural arches are the openings or foramina through which spinal nerves connect to the nerve cord.

Draw Fig. 2

3. Sternum or breast bone. *Beginning anteriorly, identify:*

Epi-sternum (thin circular cartilage)
Omo-sternum (tapered bone)
Epicoracoid cartilages (between clavicles and coracoids)
Meso-sternum (bony rod)
Xiphi-sternum (thin heart-shaped cartilage, notched behind)

4. Limb girdles (paired). *A.* Pectoral or shoulder girdle. Anterior, broadly ↄ-shaped, joined ventrally to sternum; *in either half identify:*

Suprascapula (dorsalmost, thin, hatchet-shaped)
Scapula or shoulder blade (lateral, narrowed at middle)
Clavicle* or "collar bone" (anterior, slender) } both ventral, joined to epicoracoid
Coracoid (posterior, flared at ends) } cartilages

Glenoid fossa (socket on posterior side of scapula and coracoid for head of humerus; socket + head of humerus = shoulder joint)

B. PELVIC OR HIP GIRDLE. Posterior, V-shaped, joined anteriorly to transverse process of 9th vertebra on each side; *in either half find:*

Ilium (long, slender, anterior)
Ischium (short, posterior, dorsal)
Pubis (short, posterior, ventral)
Symphysis (the median posterior fusion of the 3 pairs of bones)
Acetabulum (cup-like socket, lateral on symphysis, for head of femur; socket + head of femur = hip joint)

Draw Fig. 3

5. Paired limbs. Each long bone has a central cylindrical shaft and expanded ends (epiphyses) covered by cartilage for smooth action of the joints. *In the limbs on one side, beginning proximally, identify:*

ARM OR FORE LIMB	LEG OR HIND LIMB
Humerus (upper arm; with deltoid crest on ventral side)	Femur (thigh)
Radius + ulna, fused (forearm)	Tibia + fibula, fused (shank or lower leg)
Carpals (wrist; 2 rows of 3 small bones)	Tarsals (ankle; 2 long bones [astragalus medial, calcaneum lateral] + 2 small bones)
Metacarpals (palm; 4 and rudiment of thumb)	Metatarsals (sole; 5, long)
Phalanges (fingers; 2, 2, 3, 3)	Phalanges (toes; 2, 2, 3, 4, 3)

Draw Fig. 4

How does the skeleton of the frog compare with that of man (text, Figs. 2·9, 4·5*A*) as to general make-up? As to detailed differences? Where are hinge joints present? Which are ball-and-socket joints? What differences exist in kinds of movement possible in these two types of joints? What parts of the skeleton afford most complete protection to body parts? Why is this of advantage? Of what service is the cartilage on the ends of limb bones? Of what service are the ribs in man?

DRAWINGS

Fig. 1. Skull of frog (80 mm. long); on left side show bones seen in dorsal view, and on right side those of ventral surface.

Fig. 2. One vertebra (25 mm. long), lateral and slightly anterior view.

Fig. 3. Pelvic girdle (75 mm. long), lateral view.

Fig. 4. Entire hind limb, dorso-lateral view, as normally flexed (make femur 50 mm. long).

EXERCISE 4. THE FROG: MUSCULAR SYSTEM

(Storer, "General Zoology," pp. 24–26; 56–57; 69–70)

The two principal types of muscles in a frog are (1) the involuntary or smooth muscles of the internal organs which are studied best in microscopic sections; and (2) the voluntary or striated muscles attached to the skeleton which are under willful control and perform movements and locomotion. The latter comprise the muscular system. Each muscle is enclosed in a tough covering of connective tissue, the ***fascia,*** and many are prolonged at one or both ends by ***tendons*** of connective tissue for attachment. The ***origin*** is the more fixed or more proximal attachment, and the ***insertion*** that more movable or distal. The ***action*** of a muscle is by contraction or shortening in length, which brings the points of attachment closer together. Slender muscles often have an enlarged central part (belly), some are tapered, and others are broad and thin. Some have more than one ***head*** (origin or insertion). Of the nearly 200 muscles in a frog, many are essentially the same in name, location, and action as in other land vertebrates and in man. Each muscle is symmetrically paired on the right and left sides of the body.

Muscles seldom act singly; several usually contract together in differing amount and in sequence to produce a particular movement. Over any joint (elbow, etc.) and in other places the alternate movements are produced by more or less opposed (antagonistic) muscles.

Dissection of muscles involves freeing each from those adjacent, using a probe to loosen the fascia, so that the origin, insertion, and general action may be learned. To find deeper muscles, each more superficial muscle after identification is cut squarely across the belly. The following list gives the position, shape, origin (**O**), insertion (**I**), and action (**A**) of the principal muscles, beginning anteriorly. The smaller muscles of the eye, head, hand, and foot are omitted. *The instructor will indicate which muscles are to be dissected and the means for removing the skin.*

When removing the skin, notice that it is attached to the muscles only along certain lines, by connective tissue; the spaces between are lymph sacs. In all vertebrates except frogs and toads the skin is attached generally to the muscles. On the inner side of the frog's skin are large cutaneous blood vessels, arteries (red) and veins (dark), important in respiration.

FLOOR OF MOUTH AND TONGUE

1. *Mylohyoid* (submandibular). Broad sheet across lower jaws. **O.** Inner side of lower jaw. **I.** Tendon in median line. **A.** Raises floor of mouth in breathing. *Cut mylohyoid in mid-line and turn aside carefully to find Nos. 2 to 7.*

2. *Submental.* In tip of lower jaw, short, fibers transverse. **O.** Anterior end of mandible. **I.** Tendon in mid-line. **A.** Pushes sublingual tubercle on lower jaw against premaxillae to close external nares in respiration.

3. *Geniohyoid.* Narrow, fibers lengthwise (lateral to No. 5). **O.** Front of jaw, under submental. **I.** Divided (around No. 4); posterior process on hyoid (Fig. 10). **A.** Pulls hyoid forward, raising floor of mouth in respiration; also aids in swallowing, opening jaw, and moving tongue.

4. *Sternohyoid.* Broad (under episternum; continuation of No. 15). **O.** Dorsally on coracoid and clavicle. **I.** Ventral side of hyoid. **A.** Bulges hyoid ventrally; depresses floor of mouth in breathing.

5. *Hyoglossus.* Narrow (medial to No. 3). **O.** Posterior (thyroid) process of hyoid. **I.** The two hyoglossus muscles join, run to chin, and then into tongue. **A.** Retracts or withdraws tongue.

6. *Genioglossus.* Small, thick. **O.** Lower jaw, dorsal to submental. **I.** In tongue. **A.** Protracts or extends tongue.

7. *Petrohyoids.* Four, small. **O.** Prootic crest. **I.** Fan-like on sides of hyoid and mid-line of pharynx. **A.** Raise hyoid, in respiration and swallowing.

LOWER JAW

Remove skin over tympanic membrane and tissue between it and eye; find tympanic ring of cartilage over squamosal bone. Remove posterior end of upper jaw.

8. *Depressor mandibuli.* Behind tympanic ring; tapered. **O.** Posterior edge of tympanic ring and dorsal fascia. **I.** Extreme posterior end of lower jaw. **A.** Opens mouth.

9. *Temporal.* **O.** Side of skull between eye and tympanic ring. **I.** Near posterior end of lower jaw. **A.** Closes mouth.

10. *Masseter.* Short (between Nos. 8 and 9). **O.** Tympanic ring and adjacent bones. **I.** Lower jaw, behind temporal. **A.** Like temporal.

PECTORAL GIRDLE AND ARM

11. *Deltoid.* Anterior border of upper arm. **O.** Two heads from clavicle, scapula, and omo-sternum. **I.** Deltoid crest of humerus. **A.** Draws arm forward.

12. *Sterno-radialis* (biceps). Fan-shaped (behind No. 11, partly under No. 13). **O.** Episternum. **I.** From proximal part of radius, by tendon. **A.** Flexes forearm.

13. *Pectoralis.* Large, fan-shaped, of 4 parts. **O.** Sternum, coracoid, and rectus abdominis muscle. **I.** Converges to deltoid crest on humerus. **A.** Flexes arm; also expands abdomen by compressing viscera in thorax.

14. *Triceps brachii.* Dorsal side of upper arm. **O.** Three heads: (*a*) hind border of scapula; (*b*) anterior half of humerus; and (*c*) lateral surface of humerus. **I.** On radius, over elbow. **A.** Extends forearm.

Many small muscles on the forearm and hand act to turn and either flex or extend the wrist, palm, and fingers.

Draw Fig. 1

ABDOMINAL WALL

15. *Rectus abdominis.* Thin lengthwise sheet beside the midventral line (the whitish linea alba, of connective tissue) divided transversely by 5 tendinous bars. **O.** Pubis. **I.** Dorsally on sternum and coracoid. **A.** Supports abdomen, holds sternum.

16. *Obliquus externus.* Thin sheet over entire side of body; fibers run postero-ventrally. **O.** Dorsal fascia. **I.** Linea alba, dorsal to rectus abdominis. **A.** Supports and compresses abdomen; also compresses lung.

17. *Transversus* (+ obliquus internus). Thin sheet beneath obliquus externus; fibers run laterally and antero-ventrally. **O.** Transverse processes of 4th to 9th vertebrae, and ilium. **I.** Coracoid and xiphi-sternum, esophagus and pericardium, and on linea alba. **A.** Same as obliquus externus.

BACK AND PELVIC GIRDLE

The dorsal fascia is a strong sheet of connective tissue attached to the skull, transverse processes of the vertebrae, and ilium; it provides (and covers) the origins of several muscles. *Remove after study of No.* 18.

18. *Dorsalis scapulae* (infraspinatus). Triangular (behind No. 8, partly under No. 19). **O.** Scapula. **I.** By tendon joining that of latissimus dorsi. **A.** Raises arm toward body.

19. *Latissimus dorsi.* Narrowly tapered. **O.** Dorsal fascia. **I.** On deltoid crest of humerus. **A.** Raises arm upward and backward.

20. *Longissimus dorsi.* Along back; long and slender, with transverse septa (remove Nos. 18 and 19 to see). **O.** Urostyle, anterior third. **I.** Vertebrae and skull. **A.** Straightens back, raises head.

21. *Coccygeo-sacralis.* Narrow, fibers diagonal (behind No. 20). **O.** Urostyle, lateral middle part. **I.** Sacral (9th) vertebra, on transverse process. **A.** Singly, turns back; both muscles, raise back.

22. *Coccygeo-iliacus.* Behind No. 21; narrow, fibers diagonal. **O.** Side of urostyle. **I.** Ilium, anterior part. **A.** Holds urostyle in place.

23. *Gluteus.* Short, stout (passes ventrally between Nos. 25*a* and 25*b*). **O.** Ilium, middle of lateral surface. **I.** Anterior side of head of femur. **A.** Draws thigh forward and upward.

24. *Pyriformis.* Short, slender (behind No. 25*a*). **O.** Posterior tip of urostyle, above anus. **I.** Medial surface of femur. **A.** Raises thigh.

THIGH

When the frog's leg is extended, the lateral border (with the kneecap) corresponds to the anterior surface of the human leg. Muscles of the

thigh are described in sequence around the dorsal, posterior, and ventral surfaces.

25. *Triceps femoris.* Large, covers entire anterior border of thigh, both dorsally and ventrally. Origin by three heads joining midway on thigh:

a. Vastus externus. Dorsal. **O.** Posterior dorsal crest of ilium (behind No. 23).

b. Rectus anticus femoris. Medial, smallest. **O.** Midventral third of ilium.

c. Vastus internus. Ventral. **O.** Antero-ventral border of acetabulum.

I. Tibio-fibula, proximally, by knee tendon. **A.** Draws (adducts) thigh against trunk, also extends shank.

26. *Ileofibularis* (biceps femoris). Long, slender (between Nos. 25*a* and 27). **O.** Crest of ilium, above acetabulum (behind No. 25*a*). **I.** Two heads (*a*) distally on femur and (*b*) posteriorly on tibio-fibula. **A.** Like that of triceps femoris.

27. *Semimembranosus.* Large, postero-dorsal on thigh. **O.** Dorsally on ischium. **I.** On head of tibio-fibula posteriorly. **A.** Flexes shank.

28. *Gracilis minor* (rectus internus minor). Slender, flat, attaches to skin. **O.** Edge of ischium. **I.** Inner side of tibio-fibula, below head. **A.** Flexes shank, adducts thigh.

29. *Gracilis major* (rectus internus major). Larger than No. 28. **O, I, A,** as for No. 28.

30. *Sartorius.* Thin oblique band, across ventral surface of thigh. **O.** Ilium, below acetabulum. **I.** Inner head of tibio-fibula. **A.** Flexes shank, adducts thigh. *Cut to find No.* 31.

31. *Adductor magnus.* Large (runs under Nos. 28 and 30). **O.** Ischium and pubis. **I.** Distal third of femur. **A.** Adducts thigh and leg.

32. *Adductor longus.* Narrow (mostly under No. 30). **O.** Ilium, anterior symphysis. **I.** With adductor magnus. **A.** Same as adductor magnus.

33. *Semitendinosus.* Long, thin (*remove No.* 29 *to see*). **O.** Ischium, by 2 heads. **I.** With gracilis minor on proximal end of tibio-fibula. **A.** Adducts thigh and flexes shank.

SHANK

34. *Gastrocnemius.* Large, posterior, forms "calf" of leg (much used in physiological experiments). **O.** Two heads (*a*) distal end of femur by flat tendon; (*b*) edge of knee tendon. **I.** By Achilles tendon over heel and into plantar fascia on sole of foot. **A.** Extends foot and flexes shank.

35. *Peroneus.* Stout, posterior (between Nos. 34 and 36*a*). **O.** Knee tendon. **I.** Tibio-fibula, distally, and ankle (calcaneum). **A.**

Draws shank against thigh (before leaping or swimming), also extends or twists foot.

36. *Tibialis.* Group of 3 ventral muscles next to tibio-fibula:

a. Tibialis anticus longus. **O.** Distal end of femur by long tendon. **I.** Two heads, to proximal ends of astragalus and calcaneum. **A.** Flexes ankle.

b. Tibialis anticus brevis. Small (distally under No. 36*a*). **O.** Middle part of tibio-fibula. **I.** Astragalus. **A.** Flexes ankle.

c. Tibialis posticus. Long (under No. 34). **O.** Along entire tibio-fibula. **I.** Astragalus. **A.** Flexes and twists ankle.

37. *Extensor cruris.* Small (under Nos. 36*a* and 36*b*). **O.** Medial end of femur. **I.** Middle part of tibio-fibula. **A.** Extends shank.

There are many small muscles on the ankle, sole, and toes.

Draw Figs. 2 and 3

Find the biceps and triceps muscles of your upper arm. What is the action of each? Which is larger and why? What happens to the gastrocnemius muscle of your leg when the shank is flexed as in walking? When the leg and foot are extended? *Find the Achilles tendon above your heel.* Why is the tendon so named? When standing, what muscles of the trunk keep the body "balanced"? Which muscles do this in the shank?

How are the muscles related to joints? How few muscles will provide complete action in a hinge joint? How does a muscle "work," by shortening or lengthening? Why does it bulge when it contracts? What are some effects of exercise on a muscle? What happens to muscles during a prolonged illness?

Three orders of levers are recognized: I. Fulcrum (pivot) between power and weight. II. Weight between power and fulcrum. III. Power between fulcrum and weight. What order of lever is involved in the action of the gastrocnemius muscle of the frog? Of man? Of the triceps in the fore limb? Of the biceps in the fore limb?

DRAWINGS

Fig. 1. Muscles of chin and thorax of frog (2× natural size); show mylohyoid only on one side. Shade drawing enough to indicate paths of fibers in each muscle. Label.

Fig. 2. Muscles of extended hind leg of frog (125 mm. long), lateral view; label.

Fig. 3. Diagrammatic cross section of muscles at middle of thigh (40 mm. in diameter); label.

EXERCISE 5. THE FROG: INTERNAL STRUCTURE AND DIGESTIVE SYSTEM

(Storer, "General Zoology," pp. 19–20, 26–27, 70–80)

The ***internal organs,*** collectively termed the ***viscera,*** are contained in the large ***body cavity*** or ***coelom*** inside the trunk. In all the land vertebrates—amphibians to mammals, including man—these organs and systems are more or less alike in general structure (both gross and microscopic) and function. Study of the frog therefore affords a basis for understanding much of the anatomy and physiology of the human body. After learning the appearance and arrangement of the viscera, each organ system (text, Fig. 2·2 and p. 17) may be studied in detail.

1. Opening the body cavity. The skin and thin muscles of the ventral body wall must be cut and opened to expose the viscera, preferably using only forceps and scissors. The instructor may demonstrate how this dissection is to be made.

Lift and cut the skin across just anterior to the hind legs. Hold up the cut edge and slit the skin (only) *forward to the chin, keeping the inner blade of the scissors close up beneath the skin. Also cut the skin transversely at the fore limbs, spread it laterally, and pin out.*

Lift the thin muscular abdominal wall posteriorly and make a short cut about 3 mm. to one side of the median white or reddish line (the linea alba). *Insert one point of the scissors and cut forward at one side of the mid-line through the posterior cartilage of the pectoral girdle. Make a similar cut on the opposite side—this leaves a narrow median strip of muscle with the abdominal vein inside* (dorsally); *preserve this vein. Lift and cut the median strip anteriorly, then carefully dissect it free from the vein back to the posterior end.*

Lift and cut through the pectoral girdle (with stout scissors if available) *in the median line and continue through the muscles of the lower jaw. Gently force apart the cut margins of the girdle.*

Put the frog in a dissecting pan (or on a dissecting pad); *pin aside the edges of the body wall and also the sides of the pectoral girdle. Moisten the specimen as necessary or immerse in water.*

2. Coelom, peritoneum, and mesenteries. The coelom and the organs within it are lined by a thin smooth membrane, the ***peritoneum.*** Dorsally the peritoneum is folded downward to enclose and suspend the digestive tract and other organs (text, Fig. 2·5). Between the organs and dorsal wall the two layers of supporting peritoneum are in contact with each other and form a thin supporting ***mesentery*** in which blood vessels and nerves pass to and from organs.

The pericardium and pericardial sac around the heart are also of peritoneum, and the pericardial space is a part of the coelom separated

off during embryonic development. In a female the peritoneum is perforated by the openings of the oviducts.

The body wall surrounding the coelom consists of the skin, lymph sacs, muscles, and peritoneum.

3. Internal organs. *Beginning anteriorly, and using only the forceps and probe, identify:*

Heart (reddish, conical, muscular; enclosed within a delicate membrane, the pericardial sac)
Lungs (2, dorsal to liver, soft, thin-walled, often shriveled)
Liver (large, firm, reddish brown, of 3 lobes)
Gall bladder or bile sac (thin spherical greenish sac between middle and right lobes of liver)
Stomach (long, whitish, along left side, dorsal to liver)
Small intestine (yellowish or grayish; slender, irregularly coiled)
Large intestine or rectum (dark, passes into pelvic girdle)
Spleen (small, spherical, dark reddish, posterior to stomach)
Kidneys (2, elongate, dark brown, on dorsal wall above peritoneum)
Fat bodies (2, soft, finger-like lobes, yellowish, attached anterior to kidneys)
Ovaries (2, in female; either small and granular or distended with small black and white eggs)
Oviducts (2, in female; long, wavy, whitish, along either side of middorsal line)
Testes (2, in male; bean-shaped, pink or yellow, at antero-ventral ends of kidneys)

4. Digestive system. *A.* This system is composed of the ***digestive tract*** or ***alimentary canal*** and its associated ***glands.*** It prepares food to be absorbed into the body. *Identify the following parts, beginning anteriorly:*

DIGESTIVE TRACT

Mouth and mouth cavity with tongue and teeth (Exercise 1)
Pharynx (behind mouth cavity)
Esophagus (short, dorsal to heart; insert probe through pharynx to stomach to locate)
Stomach (whitish; large; anterior or cardiac end smaller; posterior or pyloric end larger with constriction or pyloric valve at end)
Small intestine (short anterior loop beside stomach is duodenum and receives bile duct; remainder is ileum)
Large intestine (dark; connects to cloaca)
Cloaca (within pelvic girdle; common end of digestive, excretory, and reproductive systems; see Exercise 8 for details)
Anus

DIGESTIVE GLANDS

Pancreas (irregular flattish glandular tissue, yellowish white; between stomach and duodenum)
Liver and gall bladder

Many microscopic glands occur in various organs of the digestive tract.

Draw Fig. 1

What is the function of each of the organs in the digestive system? What is the course of food in the digestive tract? Why must food be digested before it can be absorbed? What does the pancreas produce? The liver? What are feces?

B. *Trace the bile duct backward from its dorsal entrance into the duodenum. Carefully pick away the pancreatic tissue and find the duct as a whitish thread.* (It is joined by pancreatic ducts difficult to see.) *Find the cystic ducts connecting to the gall bladder and the hepatic ducts emerging from the liver to form the bile duct.*

C. STOMACH WALL (see also Exercise 1). *Make a thin cross section of the stomach by two closely parallel transverse cuts across the pyloric part with a sharp scalpel or razor blade, mount in water, and examine under low magnification; distinguish the following layers:*

Mucosa (lining of stomach cavity, contains microscopic glands)
Submucosa (thin layer of varied thickness, light to dark colored)
Muscularis (conspicuous, of uniform thickness; inner thick layer of circular muscles and outer thin layer of longitudinal muscles)
Serosa or peritoneum (extremely thin outer covering, seen only where it runs into mesentery)

In frogs that contained little or no food when preserved, the stomach will be contracted in size and the inner lining much folded.

What is the function of each of the layers named?

D. *Slit open the wall of the esophagus and small intestine to see the folds in the lining of each.* What purpose do these folds serve? What is a villus? What is a lacteal (text, Fig. 4·11)?

DRAWINGS

Fig. 1. Digestive system of frog (×2), ventral view, within an outline of the head and trunk; show lobes of liver turned anteriorly, the small intestine spread to the right, and the bladder turned to the right.

Table 1. From sources suggested by the instructor prepare a summary of human digestion, as follows:

Organ	Digestive juice	Enzyme (or activator)	Kinds of food acted upon	End product of digestion	Where absorbed
Mouth	Saliva	Ptyalin	Starches	Maltose	Small intestine
Stomach					
etc.					

EXERCISE 6. EXPERIMENTS ON DIGESTION

(Storer, "General Zoology," pp. 70–80)

Food is any substance that supplies material for the formation of protoplasm or other body parts or that yields energy for bodily activities. Food must be reduced to simple component substances by the processes of digestion before it can be absorbed —passed through the lining of the digestive tract and into the blood. These digestive processes may be duplicated by test-tube experiments with nonliving materials. The experiments may either be performed by the students or demonstrated, as the instructor directs.

1. Simple tests. ***A.*** STARCH. *Put about* 5 *ml.* (*cc.*) *of* 1 *per cent cooked starch solution in a test tube and add a drop or two of iodine solution.* The color that results is a simple test for the presence of starch. *In another tube put only* 5 *ml. of distilled water and add iodine solution*—no color should result; this is a negative test.

B. SUGAR. *Dissolve about* 1 *gram of glucose* (a simple sugar) *in* 10 *ml. of distilled water in a test tube; add about* 2 *ml. of Fehling's solution and boil;* a yellow to red precipitate indicates the presence of reducing sugar. *Make a negative test for sugar.*

2. Carbohydrate digestion. *Collect about* 1 *ml. of saliva in a test tube, add* 10 *ml. of distilled water and test for sugar; if the test is negative proceed.* A positive reaction indicates sugar in the mouth secretions, as from a meal, and will invalidate the remainder of this experiment.

A. *Number four test tubes; in each collect about* 1 *ml. of saliva and add about* 6 *ml. of starch solution.* (If necessary, chew some paraffin to stimulate salivary secretion; do not use chewing gum!) *Perform the following tests and record results:*

Begin Table 1

1. Test at once for starch.
2. Test at once for sugar.
3. Test after 30 minutes for starch.
4. Test after 30 minutes for sugar.

What do you conclude from these experiments? What happens in such digestion? What is an enzyme? What enzyme is present in saliva? What does it accomplish?

B. *In each of two test tubes* (Nos. 5, 6) *collect about* 1 *ml. of saliva, add about* 6 *ml. of distilled water, boil both, and add* 5 *ml. of* 1 *per cent starch solution to each.*

5. Test after 30 minutes for starch.
6. Test after 30 minutes for sugar.

Record the results. What do you conclude as to the heat stability of the enzyme?

3. Protein digestion. The fibrin from blood and boiled egg white are proteins such as occur commonly in animal food. *Number four test tubes* (Nos. 7 to 10) *and in each place a small* (2 mm.) *piece of protein and* 10 *ml. of distilled water; to the separate tubes add the following:*

7. Nothing.
8. Pepsin,[1] 2 ml. of 1 per cent solution.
9. Hydrochloric acid, 2 to 4 drops of 10 per cent solution.
10. Pepsin, 2 ml.; and hydrochloric acid, 2 to 4 drops.

Mix the contents of each tube thoroughly, and then either place them in an incubator at 37°C. and examine after 2½ hours, or leave at room temperature and examine at the next laboratory period. Record the results. What has happened to the protein in each? What must be present for protein digestion? Why is 37°C. selected as the temperature for incubation?

4. Emulsification of fat. During digestion, the fats present in food are in an aqueous medium in the digestive tract. In the small intestine, salts in the bile fluid are added that reduce the surface tension of the water and emulsify the fat by reducing the size of fat globules. *Place a little olive oil or other liquid fat and some water in each of two test tubes* (Nos. 11, 12); *add bile salts to the second. Examine after some minutes.* What is the result? The digestion of fats is performed by special fat-splitting enzymes not present in bile.

5. Absorption after digestion. This experiment will imitate roughly the conditions under which food is digested and absorbed. While in the cavity of the digestive tract, food is separated from the blood and lymph of the body by (*a*) the mucous membrane lining the digestive tract and (*b*) the thin walls of the blood and lymph capillaries. These are semipermeable membranes through which some substances will pass more readily than others, and some not at all. A few foods are in condition to be absorbed as eaten, but most of them, before being able to pass through these semipermeable membranes, require to be reduced by digestion to simpler chemical compounds (simple sugars, fatty acids and glycerol, amino acids). Collodion, parchment, or cellophane will serve as experimental semipermeable membranes, to demonstrate the differential absorbtion of food materials.

A. *Suspend a small sac of collodion, parchment, or cellophane in a beaker. In the beaker place some* 1 *per cent cooked starch solution and in the sac about* 50 *ml. of distilled water.*

B. *Set up another similar preparation, but add some saliva to the starch. Remove and test* 5*-ml. samples of the water from each sac separately, for*

[1] Pepsin, a protein-splitting enzyme, is extracted commercially from the inner lining of hog stomachs with dilute hydrochloric acid, filtered, and concentrated at low temperatures.

both starch and sugar, as follows: immediately, after 30 minutes, and after 1½ to 2 hours. Record the results.

Complete Table 1

The starch solution is used to represent food (undigested carbohydrate) inside the digestive tract (beaker), the sac represents the semipermeable covering of a villus of the small intestine, and the distilled water within the latter represents the blood. Actually salivary digestion stops soon after food reaches the stomach (text, pp. 75–76), and other carbohydrate-splitting enzymes take up the process in the small intestine.

TABLE 1.—RESULTS OF EXPERIMENTS ON DIGESTION AND ABSORPTION

Record as positive (+) or negative (−) in each

CARBOHYDRATE DIGESTION

	Immediately	After 30 minutes	Heated saliva
Starch	1	3	5
Sugar	2	4	6

PROTEIN DIGESTION

	Results	Conclusions
Protein	7	
Protein + pepsin	8	
Protein + HCl	9	
Protein + pepsin + HCl	10	

EMULSIFICATION OF FAT

	Results	Conclusions
Fat + water	11	
Fat + water + bile salts	12	

ABSORPTION

		Immediately	After 30 minutes	After 1½ to 2 hours
A	Starch	13	14	15
	Sugar	16	17	18
B	Starch	19	20	21
	Sugar	22	23	24

EXERCISE 7. THE FROG: CIRCULATORY SYSTEM

(Storer, "General Zoology," pp. 27–31, 80–87)

The dissections described in this exercise will show the heart and larger blood vessels, but the finer vessels and capillaries cannot be seen readily in detail. The demonstrations, together with the descriptions and illustrations in the text, will afford means for understanding their relations to the larger vessels and their importance in carrying to and from all tissues the materials essential for vital metabolic processes. Study of the circulatory system provides a basis for understanding the paths and directions of blood flow in the body.

Blood vessels are elastic but delicate; dissection involves carefully loosening and picking away their connective tissue attachments to muscles and organs with the forceps and probe. The vessels should be grasped as little as possible. Arteries contain little blood after death and usually are injected with corn starch or latex, colored red, to facilitate tracing them. Veins remain filled with blackened blood and commonly can be seen in a preserved specimen or, even better, in one freshly killed; sometimes they are injected with blue (or yellow) mass for easier dissection.

Except as stated otherwise, all arteries and veins are paired (right and left). Small variations in the relations of some vessels may occur. The blood vessels are usually dissected only on one side of the body.

1. Frog heart. ***A.*** *Examine the heart in place, as exposed by previous dissection* (Exercise 5); *identify:*

Pericardium (delicate surrounding sac of tissue)
Ventricle (posterior, conical, thick muscular walls)
Auricles (right and left, anterior, walls thin)
Sinus venosus (on dorsal side, median, posterior to auricles, thin-walled)
Truncus arteriosus (ventral, median, from anterior border of ventricle)

Draw Fig. 1

B. *Observe the beating of the heart in a freshly killed* (or anesthetized) *frog.* What is the sequence of filling and emptying of the chambers? What is the rate of heart beat? Is it affected by temperature? How does this compare with the rate in man? What is the path of blood through the frog heart? What is the pulse in man? What is blood pressure?

2. Mammalian heart. ***A.*** *Examine a demonstration of a beef or sheep heart* (which resembles closely the human heart); *identify:*

Auricles, right and left	Precaval vein	Pulmonary arteries	Aortic arch
Ventricles, right and left	Postcaval vein	Pulmonary veins	

B. *In a heart cut in frontal section to show the chambers, find the parts just listed and also:*

Bicuspid valve (between left auricle and ventricle)
Tricuspid valve (between right auricle and ventricle)
Semilunar valves (3 each, in entrances to pulmonary arch and aorta)

What is the path of blood flow in this heart? What is the purpose of the several valves?

Draw Fig. 2

3. Veins (venous system). All blood in veins flows toward the heart, but for convenience the principal vessels of the branching system of veins will be followed outward from the heart. *Trace out each vein named.*

A. Precaval vein or *anterior vena cava* (2). One enters each antero-lateral angle of sinus venosus; each is formed by union of 3 veins:

1. *External jugular vein* (most anterior), formed by
   - *a. Lingual vein,* near mid-line, from tongue and floor of mouth;
   - *b. Mandibular vein,* lateral, from lower jaw.
2. *Innominate vein,* runs dorsally; formed by union of
   - *a. Internal jugular vein,* from interior of skull;
   - *b. Subscapular vein,* from dorsal muscles of shoulder and arm.
3. *Subclavian vein* (most posterior), formed by joining of
   - *a. Brachial vein,* from arm;
   - *b. Musculo-cutaneous vein,* from muscles and skin on dorsal and lateral parts of head and trunk.

Begin Fig. 3

B. Postcaval vein or *posterior vena cava* (1). Dorsal, from between kidneys to above stomach and through liver; enters posterior end of sinus venosus; formed by confluence of

1. *Efferent renal veins* (several), medial from each kidney;

post cava also receives

2. *Hepatic veins* (2), short, from liver, just behind sinus venosus.

C. Pulmonary veins. One from each lung; both enter left auricle just anterior to sinus venosus.

D. Abdominal vein (1). Inside linea alba; joins portal vein into right lobe of liver; formed by union of

1. *Pelvic veins* (2) at posterior end of coelom; each as branch from femoral vein;

also receives

2. *Vesicular veins* (2), from bladder.

A portal system comprises veins that divide into capillaries within an organ before returning to the heart.

E. Hepatic portal vein (1). Veins from digestive organs (stomach, intestines, pancreas) and the spleen join to form the hepatic portal vein that parallels the bile duct and divides within the liver. Blood from the liver (including that from hepatic artery) leaves by hepatic veins that join postcaval vein.

F. Renal portal system. A renal portal vein runs laterally along each kidney and gives off several afferent renal veins into that organ; each renal portal vein (common iliac) is formed from

1. *Femoral vein,* from anterior side of thigh (called external iliac vein from origin of pelvic vein to junction of femoral with sciatic vein);
2. *Sciatic vein,* from posterior side of thigh;
3. *Dorso-lumbar veins* (several), from abdominal wall.

Blood from a hind leg thus may return through either the abdominal vein or renal portal vein.

Continue Fig. 3

4. Arteries. These are vessels that carry blood away from the heart. Each of the 2 long forks of the truncus arteriosus divides into 3 major vessels or aortic arches:

A. Common carotid artery (anterior arch), divides into

1. *External carotid* or *lingual artery,* to tongue and floor of mouth;
2. *Internal carotid artery* (dilated as carotid gland at its base), dorsal, to posterior roof of mouth and gives off
   - *a. Palatine artery,* on roof of mouth;
   - *b. Cerebral artery,* into cranium to brain;
   - *c. Ophthalmic artery,* to eye.

B. Pulmo-cutaneous artery (posterior arch), divides into

1. *Pulmonary artery,* short, branching onto lung;
2. *Cutaneous artery,* to skin on back and side of body and side of head.

C. Systemic arch (middle), passes dorsally beside esophagus and turns posteriorly to unite with its mate and form the ***dorsal aorta.*** Before uniting, each systemic arch gives off

1. *Laryngeal artery,* dorsal to larynx;
2. *Occipito-vertebral artery,* short, dorsal; divides into
   - *a. Occipital artery,* anterior, to side of head and jaws;
   - *b. Vertebral artery,* posterior, beside vertebral column;
   - *c. Esophageal artery,* to esophagus;
3. *Subclavian* or *brachial artery,* to arm.

Find the 2 whitish thread-like trunks of the sympathetic nervous system, one along either side of the dorsal aorta and systemic arches; avoid injuring them. Lift and cut the peritoneum to the left of the left kidney. Then lift the kidney to find the continuation of the dorsal aorta and its branches.

The dorsal aorta gives off

1. *Coeliaco-mesenteric artery* (one), which branches into
   - *a. Coeliac artery* (one), to the liver (hepatic artery) and stomach (gastric artery);

b. Anterior mesenteric artery (1), to the intestines and spleen.

2. *Urogenital arteries* (4 to 6 pairs), to the kidneys, gonads, and fat bodies;
3. *Lumbar arteries* (several), to dorsal body wall and nerve cord;
4. *Posterior mesenteric artery* (1), to rectum.

The dorsal aorta then forks into 2 *common iliac arteries;* each in turn gives off

1. *Epigastric artery,* to abdominal wall;
2. *Vesical* (recto-vesical) *artery,* to rectum and bladder;
3. *Femoral artery,* small, to anterior part of thigh.

The common iliac artery then continues as

4. *Sciatic artery,* to dorsal part of thigh, and divides to serve the shank and foot.

Complete Fig. 3

5. Lymphatic system. Vertebrates have thin-walled tubular ***lymph vessels*** of varied sizes that penetrate all tissues, collect the watery lymph, and connect to the veins (text, pp. 31, 84; Fig. 2·15). The frog also has many flat ***lymph sacs,*** large and small, between muscles and organs of the body. The more conspicuous of the latter are:

Subcutaneous lymph sacs (between skin and muscles; sacs separated by narrow septa of connective tissue)

Abdominal or subvertebral lymph sacs (above coelom between peritoneum and dorsal muscles)

Two pairs of small contractile ***lymph hearts*** pump lymph from the subcutaneous sacs into veins:

Anterior lymph hearts (under posterior corners of suprascapula over 3d vertebra)
Posterior lymph hearts (beside anus and posterior end of urostyle)

6. Capillaries. The circulation of blood through capillaries between the smallest arteries and veins can be demonstrated under a microscope in the thin foot web of a frog, or in the tail fin or gills of a larval frog or salamander.

Name in sequence the vessels and structures through which a blood corpuscle must pass in going from and returning to the ventricle if it passes to each of the following:

Tongue Lung Skin of back Intestine Kidney Hind limb

Compare blood entering the sinus venosus from the precaval vein with that from the postcaval vein as to its content of (*a*) oxygen, (*b*) nutritive materials, (*c*) nitrogenous wastes.

How are oxygenated and unoxygenated blood kept from mixing in the frog heart? In the human heart?

Where are blood cells formed? What are the functions of erythrocytes? Of white corpuscles? What is blood plasma? What functions does it serve?

What happens when an artery is cut? When a vein is cut? What stops the flow of blood from a wound?

What is the lymphatic system? What are its functions? How does lymph differ from blood?

DRAWINGS

Fig. 1. Heart of frog (50 mm. long), ventral view.

Fig. 2. Heart of mammal (50 mm. long), frontal section.

Fig. 3. Circulatory system of frog, ventral view (use entire page); outline the positions of some principal organs to aid in placing the blood vessels. Draw each vessel as two parallel lines; leave arteries clear and add cross lines for veins. Use arrows to indicate paths of blood flow.

EXERCISE 8. THE FROG: RESPIRATORY AND UROGENITAL SYSTEMS

(Storer, "General Zoology," pp. 31–34, 38–39, 87–95, 117–120)

RESPIRATORY SYSTEM

The respiratory system obtains from the environment the oxygen necessary for metabolic processes in the body and disposes of carbon dioxide, a metabolic waste. All respiratory processes involve diffusion through moist semipermeable membranes (text, pp. 47–48). Like other land vertebrates, frogs and toads have lungs for respiration. They also possess two other means of performing this function: (1) buccopharyngeal respiration, by lowering and raising the floor of the mouth, air is drawn into and forced from the mouth cavity and some O_2—CO_2 exchange occurs in the moist roof of the cavity; and (2) cutaneous respiration, through the moist skin which has many blood vessels on the inner surface (Exercises 1, 2).

1. Respiratory movements. *A. In a quiet frog* (out of water) *watch the action of the external nares, the pulsations of the mouth floor, and the occasional contractions of the thoracic wall. Determine the sequence of these several movements.* What is the rate of each? Must the mouth be closed for respiration? Why can a human being breathe with the mouth either closed or open?

B. Open the mouth of a freshly killed frog, insert a pipette in the glottis, and inflate the lungs to see their elastic nature.

2. Respiratory system. *A. Using scissors, cut through the floor of the pharynx and esophagus and around the glottis in a circle to free the larynx; then carefully dissect the lungs free from their attachments in the body so as*

to remove the entire respiratory system. Immerse in water and identify the following parts:

Glottis (slit in floor of pharynx)
Larynx or voice box (immediately ventral to glottis)
Bronchii (2; short; each bronchus connects larynx to a lung)
Lungs (2; thin-walled and elastic)

Slit the larynx midventrally and spread to see the lengthwise muscular vocal cords within. How do these cords act to produce sounds? Can a frog croak under water? Why?

Draw Fig. 1

B. *In a frog having the arteries injected with colored mass, cut a piece about* 15 *mm. square from one lung and examine the inner surface under a binocular microscope. Find the low partitions that subdivide the interior into compartments* (alveoli) *and increase the surface area; the walls contain a rich supply of fine blood vessels.*

C. *Dissect away the ventral surface of the lower jaw and find the flat hyoid cartilage embedded in the muscles* (see Fig. 10); it is moved by muscles 1–4 and 7 named in Exercise 4. How does the hyoid assist in respiratory movements?

Through what organs does air pass from the external nares to the lung? In what way is the action of lung respiration in the frog different from that in man? Of what special advantage is cutaneous respiration in a frog? Why is such respiration not possible in a reptile, bird, or mammal?

In what respects are the skin, mouth lining, and lungs in a frog alike as respiratory organs? Where and how is oxygen transported within the body? What is the difference between external and internal respiration?

UROGENITAL SYSTEM

The ***excretory system*** (kidneys, ureters, urinary bladder) disposes of liquid wastes, and the ***reproductive system*** (gonads and ducts) produces the sex cells. These two systems are structurally interrelated in the frog and other vertebrates and collectively are termed the ***urogenital system.*** All the parts are paired except the urinary bladder and cloaca.

Cut through the esophagus and across the middle of the large intestine, then remove the liver, stomach, and intestines by gently freeing them from their mesenteries; do not damage the cloaca.

3. Excretory system. ***A.*** This is alike in males and females, comprising:

Kidneys (2; each containing about 2,000 microscopic glomeruli and tubules that remove excess water and wastes from the blood stream)

Ureters (2 whitish ducts; one along posterior lateral part of each kidney to dorsal wall of cloaca; conducts the liquid urine)

Urinary bladder (1; attached midventrally to cloaca; stores urine which is voided at intervals through anus)

Ventrally on each kidney is an ***adrenal gland*** which produces internal secretions (hormones); it belongs to the endocrine "system."

B. *Examine a stained section of a kidney showing the microscopic structure.*

4. Reproductive system. Frogs are of two sexes: males producing spermatozoa, and females producing ova or eggs. *Identify the organs in one or both sexes as directed.*

MALE

Testes (2; bean-shaped, flesh-colored, each attached near kidney by short mesentery, the mesorchium)

Vasa efferentia (several minute ducts; from each testis through mesorchium to kidney)

Ureters (2; whitish duct along posterior lateral wall of each kidney to dorsal wall of cloaca)

Vestigial oviducts (2; white, wavy, lateral to ureter and kidney; present in leopard frog but not in bullfrog)

Spermatozoa pass from the vasa efferentia into microscopic uriniferous tubules within the kidney, thence into the ureter (Wolffian duct) which thus conveys both sperm and urine.

Draw Fig. 2

FEMALE

Ovaries (2; hollow lobes, small or large, the thin walls containing eggs; mesentery is mesovarium)

Oviducts (2; wavy whitish tube with fleshy walls, close to middorsal line; opens from coelom near anterior base of lung by funnel or ostium; duct often enlarged posteriorly as "uterus"; empties into dorsal wall of cloaca)

Eggs escape from the ovaries into the coelom, are moved by cilia on the peritoneum to the ostia, thence down the oviducts where glands in the walls add the jelly coats.

Draw Fig. 3

5. Cloaca. *Push the bladder to the right, then cut through the base of the left leg just to the left of the pelvic symphysis* (see skeleton for location); *cut the ligaments around the acetabulum to free the head of the femur; continue the cut through the dorsal leg muscles to the skin, and turn the leg up dorsally.*

Dissect the bladder free from the ventral body wall. Locate and cut the ileum just anterior to the pelvic symphysis, but do not damage the cloaca that lies medial. Insert a probe through the anus into the cloaca. Then slit the left side of the cloaca by following the probe, and pin the cloaca open.

Use the probe to find the opening of the bladder into the cloaca. With a dissecting microscope locate the openings of the ureters and oviducts into the dorsal wall of the cloaca.

DRAWINGS

Fig. 1. Respiratory system (×2), dorsal view.

Fig. 2. Urogenital system of male (×3), ventral view; include large intestine and cloaca.

Fig. 3. Urogenital system of female (×3), ventral view; include large intestine and cloaca.

EXERCISE 9. THE FROG: NERVOUS SYSTEM AND SENSE ORGANS

(Storer, "General Zoology," pp. 34–37, 57–58, 95–109)

The nervous system receives, stores, and transmits impulses that control and coordinate the activities of the body. Its two major divisions are (1) the central nervous system, which includes the brain and spinal cord; and (2) the peripheral nervous system, outside the preceding, which consists of the cranial nerves, spinal nerves, and sympathetic nerves and ganglia. Another division may be made between the sympathetic nervous system, which supplies the viscera, and all the other parts that comprise the cerebrospinal nervous system.

The nervous system is composed of various types of nerve cells or neurons and their cell processes. An aggregation of nerve cell bodies is called a ***ganglion*** (or in the brain, a center). A ***nerve*** consists of few or many nerve fibers (cell processes) bound together by special connective tissue. Fibers or nerves that carry impulses from receptors (sense organs, etc.) toward the central nervous system are termed afferent or sensory, whereas those carrying impulses outward, as to muscles or glands, are termed efferent or motor. Some nerves contain only one or the other type, but mixed nerves include both types. A ***nerve plexus*** is formed where adjacent nerves interchange fibers.

Nerve tissues are delicate and require great care in dissection; special stains are used to prepare sections of nervous tissues for microscopic study. Structures in the nervous system are symmetrically paired, except some in the brain.

1. Sympathetic nervous system. Parallel to the vertebrae and aorta are two white chains of small segmental ganglia connected by delicate lengthwise fibers that form the sympathetic nerve trunks. Each ganglion unites to the adjacent spinal nerve by a communicating branch (ramus communicans). Fibers extending out from the ganglia unite to form plexes, then distribute to the internal organs, glands, and blood vessels. Many of these nerves have been removed in earlier dissections. The splanchnic nerve, along the coeliaco-mesenteric artery, goes to the solar plexus, whence fibers distribute to the stomach and near-by organs; relics of this nerve may usually be seen.

2. Spinal nerves. *Remove the heart and lungs by tearing the mesenteries to which they attach.* In the dorsal wall of the coelom the spinal nerves emerge from between the vertebrae and appear as white cords (sometimes yellowish in preserved specimens). Each arises from the spinal cord by two roots, ventral and dorsal, and each dorsal root has an external ganglion which is surrounded and hidden by a white calciferous body.

Locate and trace the spinal nerves; each emerges behind the vertebra of the same number:

First (runs latero-ventrally to muscles in floor of mouth)
Second or brachial (largest, at level of arm; branches into shoulder, breast, and arm)
Third (to muscles of body wall)
} Branches interconnect (anastamose) to form the brachial plexus

Fourth (slender; passes obliquely backward to ventral wall of abdomen)
Fifth (same)
Sixth (same)
Seventh (large; run almost directly backward)
Eighth (same)
Ninth (same)
} Anastomose to form sciatic plexus; 8th and 9th join to form the sciatic nerve to hind limb

Tenth (minute; emerges from openings anteriorly in urostyle; innervates urinary bladder and cloaca)

Draw Fig. 1

Why is the second spinal nerve so large? What is the purpose of the brachial plexus? Of the sciatic plexus?

3. Central nervous system. *Strip off the middorsal skin from the head and back and the muscles over the vertebrae. Make a short lengthwise cut just medial to each external naris, then cut across between the nares; lift and turn back the cartilage. With scissors and forceps, chip away the roof of the cranium in small pieces, keeping the inner point of the scissors close to the bone so as not to injure the soft brain just within; continue until the entire brain is exposed. Then snip both sides of each neural arch and remove the dorsal pieces until the spinal cord is laid clear.*

Two thin pigmented membranes, the ***meninges,*** surround the central nervous system: (*a*) the dura mater, which usually adheres to the cranial wall and neural canal, and (*b*) the pia mater, which closely covers the brain and cord.

4. Brain. *A.* DORSAL SURFACE. *Beginning anteriorly, identify:*

Olfactory lobes (2; spherical, separated by faint median groove; from each the first cranial or olfactory nerve branches onto the nasal sac)
Cerebral hemispheres (2; slender, tapered, with furrow between; closely joined to olfactory lobes)
Diencephalon (median, behind and below hemispheres; covered by anterior choroid plexus of blood vessels)
Pineal body or epiphysis (slender stalk, knobbed at end, from diencephalon dorsally to "brow spot"; often lost in dissection but scar of base remains)
Optic lobes (2; ovoid, diverge behind diencephalon; form dorsal part of midbrain)

Cerebellum (narrow transverse ridge behind optic lobes; much smaller than in other vertebrates)
Medulla oblongata (ventral, tapers to join spinal cord; dorsal surface with narrow wedge-shaped opening covered by posterior choroid plexus, a network of blood vessels in a delicate membrane)

Draw Fig. 2

B. VENTRAL SURFACE. *Working under water, cut across the olfactory nerves and begin to turn the brain up and backward; preserve the fine roots of the cranial nerves so far as possible. When free ventrally, cut across just posterior to the medulla to free the brain from the spinal cord. On the ventral surface find:*

Optic chiasma (crossing of the large second cranial or optic nerves ventrally on diencephalon)
Infundibulum (broad, thin, bilobed, posterior to chiasma)
Pituitary body or hypophysis (an endocrine gland, continuous with infundibulum; often remains in floor of cranium when brain is removed)
Cranial nerves (paired roots below optic lobes on midbrain and on sides of medulla)

Draw Fig. 3

C. CAVITIES OF THE BRAIN. *Use a razor blade or sharp scalpel to cut thin slices from the dorsal surface of the brain until the internal cavities are exposed. Identify:*

First and second ventricles (in cerebral hemispheres)
Third ventricle (in diencephalon)
Foramina of Munro (connecting 1st and 2d ventricles with 3d ventricle)
Optic ventricles (in optic lobes; not numbered since none occur in mammals)
Aqueduct of Sylvius (slender duct in lower part of midbrain; connects to optic ventricles and between 3d and 4th ventricles)
Fourth ventricle (long cavity in medulla)

Draw Fig. 4

What is the function of each of the parts of the brain? How does the brain of the frog compare with that of man and other vertebrates as to relative size? (See models; also text, Figs. 4·22, 4·26.) As to size of the various parts? What parts are proportionately larger in the human brain?

5. Spinal cord. ***A.*** *Cut down the remaining sides of the neural arches to expose the roots of the spinal nerves; identify:*

Anterior or brachial enlargement of cord
Posterior or lumbar enlargement (cord then tapers to filament in urostyle)
Dorsal and ventral roots of spinal nerves (paired; emerge laterally through intervertebral foramina between neural arches)

Around the dorsal root ganglion of each nerve is a dense white ***calciferous body.***

B. Lift the anterior end of the cord and cut a thin cross section; or examine a stained cross section of the spinal cord. Locate:

Longitudinal fissures (dorsal and ventral)
Central canal
Motor neurons of ventral horn ganglia
White matter (fibers)
Gray matter (with nuclei)

Draw Fig. 5

What is the difference in function between the dorsal and ventral roots of a spinal nerve? What are the structural differences between the white matter and gray matter of the cord? What is a reflex arc? What is the path of nerve impulses in a reflex action? How does a reflex action differ from a voluntary action? What are some common reflexes in the human body?

6. The ear. *A.* If not removed previously, *cut the skin around the eardrum and carefully peel it off.* This exposes the tympanic ring covered by the delicate circular tympanic membrane. *Cut around this membrane and find within the middle-ear cavity containing a slender bone, the columella.* Sound waves in air or water set the tympanic membrane into vibration. From the thickened central part of the membrane, the columella transmits these vibrations within the skull to register on sensory endings of the auditory nerve in the inner ear. *Probe the middle-ear cavity and find its connection to the mouth cavity by the Eustachian tube;* this enables the air pressure on the two sides of the tympanic membrane to be equalized.

B. Examine a model or diagram of the human ear; also a dissection of the inner ear of a frog or shark. Study these to determine the structural features of various parts. In the inner ear, identify:

Semicircular canals (3; in planes at right angles to each other; each with swelling or ampulla containing nerve endings)
Utriculus, Sacculus } in shark or frog
Cochlea (in mammal)

Where is the tympanic membrane in man? What is the function of the fleshy external ear (pinna) in man and mammals? What are the semicircular canals? What are the two distinct functions of the inner ear?

7. The eye. *A. Remove both eyeballs by cutting each around the lids, then through the optic nerve and the muscles attaching to the eye socket or orbit of the skull. Identify:*

Eyelids (upper, thick; lower, thin; nictitating membrane or 3d eyelid, inside the others)
Conjunctiva (smooth membrane lining eyelids and covering cornea)
Cornea (transparent front or lateral surface of eyeball)
Iris diaphragm (thin pigmented membrane behind cornea)
Pupil (central oval opening in iris through which light enters eye)

Optic nerve (whitish; enters back or medial surface of eyeball)
Sclerotic coat (tough outer membrane covering eyeball; continuous with cornea)

B. *With a sharp instrument bisect one eyeball in a vertical plane through the optic nerve and pupil; cut the other midway from lateral to medial surfaces, at right angles to the plane used for the first. Beginning externally, identify:*

Anterior cavity (between cornea and iris; filled with watery aqueous humor)
Lens (behind iris; firm flattish sphere; transparent in life)
Posterior cavity (behind lens; filled with gelatinous vitreous humor)
Retina (delicate innermost lining of eyeball; contains microscopic rods and cones of vision from which nerve cells connect to fibers in optic nerve; retina sometimes loosened in preserved specimens)
Choroid coat (blackened layer between retina and sclerotic coat; contains many blood vessels and much pigment)

Draw Fig. 6

C. *If available, examine a demonstration specimen of the eye of a shark or of some mammal and note the three opposed pairs of muscles that rotate the eyeball.* A sectioned mammalian eye will show also the ciliary muscles, which change the curvature of the lens for focusing on near objects. *Examine a model of the human eye if available.*

What field of vision does a frog possess? Can it see directly behind its body? Is the frog's vision monocular or binocular? What is the function of each of the parts of the eye named in Pars. 7A and 7B above? How do these parts compare with those of a camera in producing an image on the sensitized film? *Remove the lens from an eye and try its use as a magnifier.*

DRAWINGS

Fig. 1. Spinal nerves and sympathetic trunks (150 mm. long), ventral view; number the spinal nerves; show details of the brachial plexus.

Fig. 2. Brain (75 mm. long), dorsal view; label fully.

Fig. 3. Brain (75 mm. long), ventral view; label all parts seen.

Fig. 4. Brain (75 mm. long), frontal section, showing cavities; stipple the brain tissue lightly and leave the cavities clear; label parts of brain and the cavities.

Fig. 5. Cross section of spinal cord (50 mm. wide); label parts named in Par. 5B.

Fig. 6. Eye (70 mm. in diameter), in median vertical section; label parts named in Par. 7.

EXERCISE 10. THE FROG: NERVOUS RESPONSES

(Storer, "General Zoology," pp. 95–96, 101–109)

Every living organism, animal or plant, is constantly being subjected to various influences by its environment. Some of these are external in origin, and others arise within its body. Any change in either the external or internal environment that is capable of bringing about a response is called a ***stimulus.*** The ability of living matter to respond or react to stimuli is termed ***irritability.*** The responses may be either local or general, affecting one or a few parts or else the entire organism. The stimuli act upon special structural units known as ***receptors*** (sense organs in some cases); these in turn induce ***nerve impulses*** that travel through various parts of the nervous system finally to reach terminal ***effectors*** on muscles or glands that produce the responses.

Study of nervous responses is made by applying various kinds of stimuli to animals and observing the results. Such experiments may be either shown as demonstrations or performed by students, as the instructor directs. Inferences may be drawn as to the purpose and advantage of each type of response in the life of the frog. By destroying certain parts of the nervous system and then applying stimuli, information may be obtained on the localization of some functions within the nervous system. Many additional experiments are possible on various bodily functions in a laboratory equipped with special apparatus for physiological studies.

ENTIRE FROG

Each experiment (A to F) will employ frogs in four different conditions with respect to the central nervous system:

I. Normal (uninjured)
II. Cerebral hemispheres (cerebrum) and diencephalon removed
III. Entire brain destroyed.
IV. Entire brain and spinal cord destroyed.

Apply in turn to each frog (I to IV) *the stimuli listed below* (A to F). *In each case note the kind and intensity of the stimulus and the nature and degree of the response. When possible, determine the time* (in seconds) *elapsing from application of a stimulus until a response* (muscular movement) *occurs. Record the results.*

Begin Table 1

1. Local reactions. *Apply the following stimuli:*

A. TOUCH. *With forceps pinch a hind toe gently, then firmly.*

B. ELECTRIC SHOCK. *Apply the electrodes from the secondary of an induction coil (adjusted for a light shock) to a hind foot and close the switch key for an instant.*

C. HEAT. *Bring a hot iron rod close to some part of the trunk or a limb.*

D. CHEMICAL. *Moisten a small piece of paper* (2 to 3 mm. square) *in* 10 *per cent acetic acid and apply in turn to the skin on different parts of*

the body (abdomen, back, arm, hind leg, toe). *After each test wash the skin well with water.*

Does the part stimulated respond in each case? Are there differences between the four frogs? On what parts are the responses most rapid? Why?

2. Coordinated responses. *In the next two experiments observe the kind of response and the body parts involved:*

E. RIGHTING THE BODY. *Turn the frog on its back and see whether the animal can regain its normal position.* What body parts are involved in the response? Through what parts of the nervous system is such coordination established?

F. COMPENSATORY MOVEMENT. *Place the frog in a small glass dish. Rotate the dish first in one direction and then another; also tilt the dish from side to side.* What special organs related to the nervous system are involved in maintaining equilibrium?

In general, what may be concluded as to the role of the several parts of the nervous system from the experiments on frogs II to IV?

What is a reflex? What is a reflex arc? What are the structural parts of a reflex arc?

NERVE-MUSCLE PREPARATION

The effects of direct stimulation may be studied conveniently by use of the gastrocnemius muscle and sciatic nerve prepared as follows:

On a freshly pithed frog cut the skin around the pelvis and strip it off each leg; avoid touching the exterior of the skin to the muscles because the skin secretions will irritate the muscles. Lay the frog dorsal side up on a carefully washed glass plate, and keep the muscles moistened with 0.7 *per cent salt solution.*

On the dorsal surface of the thigh, part the ileofibularis and semimembranosus muscles to find the sciatic nerve as a stout white cord paralleling a vein. Use a clean glass hook to free the nerve (do not stretch or pinch it) *back to the spinal cord and cut the nerve there.*

Then dissect out the gastrocnemius muscle on the shank by (1) *cutting the Achilles tendon on the ventral surface of the foot,* (2) *removing the tibiofibula and its adhering muscles at the knee,* (3) *removing other muscles attached to the femur, and* (4) *cutting across the femur about an inch above the knee. This will free the gastrocnemius muscle with its two heads attached to the distal end of the femur.*

Fasten the femur in a clamp on a ring stand; lay the sciatic nerve on moist filter paper on a small glass plate attached to the stand, and hook the Achilles tendon to a muscle lever by a fine S*-shaped wire.* The fine tip of the muscle lever may be brought against smoked paper on the rotating drum of a kymograph to provide a record of the responses.

3. Nerve stimulation. *In turn apply each of the following stimuli to the free end of the sciatic nerve; observe and record the results. After each experiment cut off a short piece of the nerve before proceeding.*

A. TOUCH. *Pinch the end of the nerve with forceps.*

B. ELECTRIC SHOCK. *Apply the electrodes from the secondary of an induction coil.*

C. HEAT. *Apply a heated rod of glass or iron to the end of the nerve, but keep the heated rod away from other parts.*

D. CHEMICAL. *Place a few salt crystals* (NaCl) *against the cut end of the nerve, and wait a few minutes for the response.*

4. Direct stimulation of muscle. *In turn apply each of the stimuli* (A to D) *directly to the muscle; observe and record the results.*

Complete Table 1

What evidence shows that an impulse passes along the nerve? Why are different kinds of stimuli able to induce a similar response? What is the nature of the nerve impulse? If the muscle responds to direct stimulation, what does this indicate as to the properties of the protoplasm in muscles?

TABLE 1.—NERVOUS RESPONSES IN FROGS

For each numbered item (1 to 32) record both the result observed and the conclusions

ENTIRE ANIMAL

Stimulus	Condition of central nervous system			
	I Normal	II Cerebrum and diencephalon removed	III Entire brain destroyed	IV Entire brain and spinal cord destroyed
LOCAL				
A. Touch	1	2	3	4
B. Electric shock	5	6	7	8
C. Heat	9	10	11	12
D. Chemical	13	14	15	16
GENERAL				
E. Body inverted	17	18	19	20
F. Body rotated and tilted	21	22	23	24

NERVE-MUSCLE PREPARATION

Stimulus	Part stimulated	
	Nerve	Muscle
A. Touch	25	26
B. Electric shock	27	28
C. Heat	29	30
D. Chemical	31	32

EXERCISE 11. MITOSIS

(Storer, "General Zoology," pp. 48–51)

All cells arise from preexisting cells, principally by a process of indirect cell division known as ***mitosis.*** Cells thus multiply to bring about the development of a new individual animal from a fertilized egg, the transformation from larva to adult as in a frog, the growth of body parts, and the repair of injuries. In mitosis, division of the nucleus precedes that of the cytoplasm (cytosome). The nuclear changes comprise a continuous process, structural and physiological, but for convenience in study they are divided into several stages or phases: ***prophase, metaphase, anaphase,*** and ***telophase.***

Mitosis is a universal phenomenon in animals and plants, and can be observed in all kinds of cells, but is studied most readily in large cells having few chromosomes and undergoing frequent division. Favorable animal materials are (1) eggs of *Ascaris megalocephala*, a large intestinal roundworm of the horse, with four chromosomes; (2) eggs of whitefish (*Coregonus*); and (3) the epidermal cells of salamander larvae; both of the latter have more chromosomes. Among plant materials, the growing root tips of onion and of *Tradescantia* often are used.

Microscopic sections of *Ascaris* eggs are prepared from female worms (see Exercise 32); the uteri are removed, cut into short lengths, fixed, embedded, and sectioned (usually at 10 μ in thickness); several cross or longitudinal sections from different parts of the uterus may be mounted on each slide and stained to show various stages in mitosis (and meiosis; Exercise 12). Since the entire egg cells are large, microscopically, each egg is cut into several "slices"; purely by chance, some of these will contain entire mitotic figures, others only parts of figures, and some only cytoplasm. In a suitable section the entire mitotic figure will be seen in a lateral or "equatorial" view, whereas a "polar" view (along the axis of the mitotic figure) will show the chromosomes as arranged in the plane of the cell "equator."

1. Ascaris eggs. ***A.*** *The instructor will indicate which section on the prepared slides will contain eggs undergoing mitosis. Examine first under medium power of the compound microscope.* The outermost thick wall of the uterus is lined with large irregular epithelial cells. Inside the uterus are many rounded ***eggs,*** each consisting of an ***egg cell*** with its ***cell membrane*** and surrounded by a ***shell;*** the latter has a thin dark covering and thick translucent lining. In prepared sections, the protoplasm often shrinks to leave a clear ***perivitelline space*** between the cell membrane and shell. Small dark-colored (polar) bodies may be present on the cell membrane of some eggs; they will be considered in Exercise 12.

B. *Under high magnification give attention to the cells and their contents.* Some egg cells will be undergoing mitotic division from the 1- to 2-cell or 2- to 4-cell stage, and others will be in the "resting" or nondividing condition. *Find examples of each phase in mitosis* (Pars. 3–6), *and determine the form and condition of the cell components in each. Consult demonstrations for any phases not found readily. From this study be able to visualize clearly the entire process of mitosis.*

2. "Resting" cell. *In a cell not undergoing mitosis, identify:*

Cell membrane (thin, entirely surrounding cell contents)
Cytoplasm (within cell membrane, vacuolated)

Nuclear membrane (thin, separates contents of nucleus from cytoplasm)
Chromatin network (irregular short dark-staining threads and dots within the nucleus)
Nuclear matrix or nucleoplasm (filling spaces between network)
Centrosphere (small clear sphere in cytoplasm, usually close to nucleus)
Centrosome (dark-staining dot or granule within centrosphere)

The centrosome and chromatin of the nucleus usually are stained dark blue or blackish with hematoxylin, a nuclear dye. The cytoplasm often is grayish; it is pink or reddish if stained with eosin or some similar cytoplasmic dye.

Draw Fig. 1

3. Prophase. This stage comprises all changes during mitosis up to the splitting of the chromosomes, including (*a*) division of the centrosome into two which separate to the "poles" of the cell on opposite sides of the nucleus; (*b*) gradual disappearance of the nuclear membrane; (*c*) formation of the several distinct chromosomes by condensation of the chromatin material; the chromosomes become arranged in the equatorial plane of the cell which is at right angles to the axis between the two centrosomes; (*d*) appearance of short radiating rays forming an "aster" about each centrosome; and (*e*) appearance of a spindle of "fibers" between the centrosomes and chromosomes.

Examine cells in early and late stages of the prophase and identify all the parts mentioned.

EARLY PROPHASE OR SPIREME STAGE. Chromatin network forming into long threads that stain darkly.

LATE PROPHASE. Chromatin material aggregated into four distinct chromosomes.

Draw Figs. 2, 3, and 4

4. Metaphase. Each chromosome splits lengthwise into two equal and equivalent parts. The metaphase is of short duration and may be difficult to find. *Examine the cell in lateral view.*

Draw Fig. 5

5. Anaphase. The two sets of equivalent ("daughter") chromosomes separate toward opposite "poles" (centrospheres), and spindle fibers appear between each pair of chromosomes. The cytoplasm and cell membrane begin to constrict between the two sets of chromosomes. *Study in lateral view.*

Draw Fig. 6

6. Telophase. In the terminal phase of mitosis, (*a*) the chromosomes approach the centrosomes, cluster, and begin to break apart; (*b*) a nuclear membrane forms about each chromatin mass; and (*c*) constriction of the cytoplasm and growth of the cell membrane continues until

the two "daughter" cells are entirely distinct. Mitosis then is complete. *Study in lateral view.*

Draw Fig. 7

What materials in the "resting" cell provide the substance of the densely staining chromosomes present during mitosis? What hereditary components are the chromosomes believed to carry? Of what significance is the equal quantitative and qualitative division of the chromosomes during mitosis? What is the theory of "the individuality of the chromosomes"? Is there evidence from mitosis in *Ascaris* to support this theory?

DRAWINGS

Arrange the following figures in two vertical columns, making each figure about 50 mm. in diameter. To save time, draw each stage as found. Omit the uterine wall and egg shell. In each figure label the following where they appear:

Cell membrane	Aster
Cytoplasm	Astral rays
Nuclear membrane	Centrosphere
Chromosomes	Centrosome
(or chromatin)	Spindle fibers

Fig. 1. Resting cell.

Fig. 2. Early prophase (spireme).

Fig. 3. Late prophase; entire mitotic figure in lateral or equatorial view.

Fig. 4. Late prophase with equatorial plate of chromosomes in polar view.

Fig. 5. Metaphase, lateral view; show spindle arrangement and splitting of chromosomes.

Fig. 6. Anaphase, lateral view.

Fig. 7. Telophase, lateral view.

EXERCISE 12. MATURATION, MEIOSIS, AND FERTILIZATION

(Storer, "General Zoology," pp. 115–125)

Sexual reproduction[1] in animals involves the union of two sex or germ cells of unlike kind, a male cell or spermatozoan (sperm) with a female cell or ovum (egg). These are produced in and set free from the sex organs, the testis and ovary, respectively. In their early stages, the germ cells multiply by mitosis; later they undergo special changes known as ***maturation*** or ***gametogenesis*** to become mature sex cells or ***gametes.*** This process is termed ***spermatogenesis*** in the male and ***oögenesis*** in

[1] Several types of asexual reproduction are illustrated by fission in amoeba (Exercise 18), sporulation in sporozoans (Exercise 21), and budding in hydra and other coelenterates (Exercises 25, 26).

the female. During maturation the chromosomes divide only once, but there are two segregations, the ***first and second meiotic divisions.*** As a result, the number of chromosomes in each germ cell becomes reduced from the diploid number ($2n$), which is characteristic of somatic or body cells and early germ cells, to the haploid number (n) of mature gametes. This "reduction of the chromosomes" is termed ***meiosis.***

The gametes of the two sexes in any species of animal differ in size, form, and physiology, but the meiotic changes in their nuclei are essentially comparable.

The entrance of a mature sperm into a mature egg is termed ***fertilization.*** Fusion of their nuclei results in a fertilized egg or ***zygote*** which has the diploid number of chromosomes ($n + n = 2n$). This is the starting point for the development of a new individual (Exercise 13).

As a result of the segregation of chromosomes during meiosis, each gamete (either ovum or sperm) has either one or the other chromosome from each homologous pair; thus gametes differ in their chromosomal content. In fertilization, there is chance meeting of egg and sperm, affording additional opportunity for different combinations of chromosomes to be brought together in any zygote. There is much evidence to indicate that the chromosomes carry the factors (genes) responsible for the development of hereditary characteristics in offspring. The random segregation and recombining in maturation and fertilization afford an explanation of the hereditary variations between the offspring from the same or different parents. A clear knowledge of the nuclear phenomena during meiosis and fertilization and of their significance is essential to understanding the processes of heredity and finds practical application in the genetics of animal and plant breeding.

SPERMATOGENESIS

The entire maturation process of male germ cells is completed before they are released from the testis, including both meiotic divisions and the transformation into the physical form of spermatozoa. Favorable material for study is found in stained sections of the testes of salamanders or grasshoppers taken in the seasons when spermatogenesis is in progress. The number of chromosomes in these animals is larger than in *Ascaris*. Not all stages of each meiotic division will necessarily appear on any one prepared slide.

A salamander testis is composed of many distinct spherical lobes, with all germ cells in each at about the same stage in maturation. Lobes at one end (anterior) contain the earliest stages (spermatogonia) and those toward the opposite end, opening into the sperm duct, contain mature spermatozoa. The meiotic divisions commonly are present only in a few lobes, whereas several will contain spermatids in various stages of metamorphosis into spermatozoa.

The testis of a grasshopper consists of many small slender cylindrical lobes, each opening directly into the sperm duct; for the purpose of this study, any one lobe corresponds to the entire testis of a salamander since each is subdivided into minute lobules. Early stages in spermatogenesis are found in lobules farthest from the duct, mature spermatozoa in those near the duct, and transitional stages in others between.

1. Spermatogenesis. *Identify the following stages* (A to E) *and study the details in each under high magnification:*

A. SPERMATOGONIUM. The cell is small and round, with indistinct boundaries and pale cytoplasm; the chromatin in the nucleus is dispersed as a dark-staining network or reticulum.

Draw Fig. 1

B. PRIMARY SPERMATOCYTE. This is derived from the spermatogonium by increase in size; the chromatin network stains more heavily.

Draw Fig. 2

FIRST MEIOTIC DIVISION

Prophase. The chromosomes are stout and conspicuous, staining intensely; those of each homologous pair come close together (***synapsis***); then both in each pair split longitudinally but do not separate, thus forming ***tetrads*** (packets of 4). Temporarily the nucleus contains $4n$ chromosomes. The tetrads first are single loops, then shorten and assume various shapes (∩, L, T, etc.), finally becoming aligned in the equator of a division spindle between the 2 centrosomes.

Metaphase. Each tetrad splits into 2 ***dyads*** (each of $2n$ chromosomes).

Anaphase. The 2 dyads from each tetrad move toward opposite centrosomes.

Telophase. Two cells or ***secondary spermatocytes*** result (each contains $2n$ chromosomes); nuclear membranes form, the chromatin within each becomes a reticulum, and a cell membrane separates the 2 cells.

C. SECONDARY SPERMATOCYTE. The diameter of the nucleus and cytoplasm in each is about 0.8 of that in the primary spermatocyte.

Draw Fig. 3

SECOND MEIOTIC DIVISION

The centrosome soon divides into 2.

Prophase. The chromosomes of the dyad reappear in their former shapes and become aligned on the equatorial plate.

Metaphase. Each dyad separates into 2 distinct chromosomes.

Anaphase. The 2 chromosomes from each dyad move toward opposite poles.

Telophase. From each secondary spermatocyte, 2 ***spermatids*** result (each is a "monad" containing n chromosomes); the chromatin in each nucleus returns to a reticulum within the newly formed nuclear membrane.

Draw Fig. 4

D. SPERMATID. Four spermatids thus result from one primary spermatocyte, the diameter of each being about 0.6 (0.25 of the volume) of the primary spermatocyte. By a metamorphosis or change in shape (spermiogenesis), each spermatid becomes transformed into a still smaller ***spermatozoan.*** In this change the centrosome divides into two; one remains close to the nucleus to produce the anterior end (perforatorium or acrosome) of the sperm, and the other migrates to the opposite side of the nucleus and sends out a long slender filament (flagel-

lum) surrounded by a thin layer of cytoplasm. The nucleus is condensed to a small homogeneous mass, and much of the cytoplasm is cast off.

Draw Fig. 5

E. SPERMATOZOAN. The mature male gamete or spermatozoan consists of (1) the ***head,*** composed of the acrosome and the dark-staining nucleus; (2) a ***mid-piece*** from which the flagellum arises; and (3) the long slender ***tail*** composed of the flagellum and its sheath.

Draw Fig. 6

OÖGENESIS

Eggs of the nematode worm, *Ascaris,* are favorable material for the study of oögenesis and fertilization since the chromosomes are few and large. Prepared slides usually contain sections from several different parts of the uterus of the worm to show various stages in these processes. Such sections are obtained as described in Exercise 11. Of the stages described below, the oögonia and early primary oöcytes can be seen only in sections of the ovary, where synapsis and tetrad formation take place. The first stage usually present on student slides is the late prophase of the first meiotic division. In *Ascaris,* oögenesis stops at this phase until after a sperm has entered the egg cytoplasm. The sperm has a peculiar shape, unlike that of most animals (text, Fig. 5·8). The sperm nucleus becomes a large black dot centered in the cytoplasm, and the remainder of the sperm gradually disappears. Soon after sperm entry a shell forms about the oöcyte. The egg of *Ascaris,* like those of most animals, thus acquires most of its physical characteristics before maturation.

2. Oögenesis. *Study a prepared slide, identify the stages in maturation present, and examine the details in each carefully under high magnification. The instructor will indicate which sections are to be studied.*

A. OÖGONIUM. The cells multiply by mitosis in the ovary and are small, with pale cytoplasm and a small nucleus having a densely compacted chromatin network.

B. PRIMARY OÖCYTE. This larger stage is derived from the oögonium by addition of food (yolk) to the cytoplasm; the nucleus is enlarged and the chromatin network is conspicuous.

FIRST MEIOTIC DIVISION

Prophase. The chromatin network resolves into 4 densely staining chromosomes that become arranged in 2 homologous pairs; this process is termed ***synapsis*** or "pairing of the chromosomes." (Of the 2 in each homologous pair, 1 derived from the male and 1 from the female parent of the previous generation.) The chromosomes shorten and thicken, then both in each pair split longitudinally but do not separate thus forming ***tetrads*** (packets of 4). Temporarily the egg nucleus contains 8 ($4n$) chromosomes. The 2 tetrads then move toward the periphery

of the oöcyte and become aligned on the equator of a division spindle between the 2 centrosomes.

Draw Fig. 7

Metaphase. Each tetrad divides into 2 ***dyads*** (of 2 chromosomes each).

Draw Fig. 8

Anaphase. / *Telophase.* Unequal division of the cytoplasm follows. Two dyads (one from each tetrad) separate in a minute cell, the ***first polar body,*** on the surface of the egg. The remainder of the nuclear material, together with the egg cytoplasm, becomes the ***secondary oöcyte.*** The nucleus of the latter and that of the first polar body each contain 4 chromosomes ($2n$).

Draw Fig. 9

C. Secondary oöcyte. This cell is of the same size as the primary oöcyte. The chromosomes do not return to a "resting stage," but each pair of dyads rotates 90 degrees so as to lie opposite the other pair, thus ⁚⁚

Draw Fig. 10

Second Meiotic Division

This follows shortly and in the manner of the first division.

Prophase. A division spindle forms about the 2 dyads of the oöcyte.

Metaphase. The 2 members of each dyad separate, as single chromosomes (monads), to opposite poles of the division spindle.

Anaphase. / *Telophase.* Two chromosomes (one from each dyad) are extruded in a small membrane on the surface of the oöcyte as the ***second polar body.*** The egg nucleus together with the cytoplasm constitutes the ***oötid.*** The second polar body and the oötid nucleus each contains 2 chromosomes (n).

Draw Fig. 11

D. Oötid. A nuclear membrane forms, within which the 2 chromosomes become a chromatin network; this is the ***pronucleus*** of the mature ovum or female gamete. Both polar bodies later degenerate and disappear.

MATURE GAMETES

3. Mature gametes. ***Examine demonstrations of the ova and spermatozoa of a frog to see the differences in the form and size of mature gametes. If available, see also a fresh preparation showing live, moving spermatozoa.***

This may be made by taking small pieces of the testis from a freshly killed frog, teasing them apart in water on a slide, and laying on a coverglass. Reduced illumination is desirable. See also the gametes of any other animals that may be demonstrated.

FERTILIZATION

4. Fertilization. In different species of animals, the entrance of the sperm into the egg occurs at different stages in the maturation of the latter. In *Ascaris*, following maturation of the egg, the sperm nucleus becomes transformed into a ***pronucleus,*** identical with that of the mature ovum, and the two come close together in the center of the egg.

Draw Fig. 12

The membranes about the 2 pronuclei disappear, and the chromatin in each becomes organized as 2 chromosomes. A division figure (mitotic) forms with the 4 chromosomes ($2n$) in an equatorial plate. This is the ***fertilization nucleus,*** and the egg thus fertilized is termed a ***zygote*** (Gr. *zygos*, yoke; chromosomes from the male and female thus being yoked together). Henceforth, by mitotic divisions, there results a developing egg, an embryo, and eventually a new individual animal (Exercise 13; text, pp. 127–138).

Draw Fig. 13

Which portion of a hen's egg is the female gamete? How do male and female gametes differ as to physiology? What are the two principal results of maturation? What events in maturation provide for variation in the characteristics of offspring? In what respects does oögenesis differ from spermatogenesis? In what respects are the two processes alike?

DRAWINGS

Spermatogenesis

For each figure, outline 4 or 5 cells and their nuclei; then in one show the nuclear details clearly, and label. Make the primary spermatocyte about 30 mm. in diameter and the other stages in proportion.

Fig. 1. Spermatogonium (if present).
Fig. 2. Primary spermatocyte.
Fig. 3. Secondary spermatocyte.
Fig. 4. Spermatid.
Fig. 5. Several stages in metamorphosis of spermatid into spermatozoan.
Fig. 6. Mature spermatozoan (about 75 mm. long).

Oögenesis

For each figure, outline the cytoplasm of one egg (about 50 mm. in diameter, omit shell); show the nuclear details clearly and label fully.

Fig. 7. Late prophase of first meiotic division with tetrads.
Fig. 8. Metaphase of first meiotic division.
Fig. 9. Separation of first polar body (telophase).
Fig. 10. Rotation of dyads.
Fig. 11. Separation of second polar body.

Fertilization

Fig. 12. Pronuclei, male and female, in center of cytoplasm.
Fig. 13. Fertilization nucleus.

EXERCISE 13. EMBRYOLOGY

(Storer, "General Zoology," pp. 40–41, 127–133, 133–135)

Embryology is the branch of zoology that deals with the development of new animals from eggs. The egg is a cell; in different species its cytoplasm contains various amounts of yolk that will serve to nourish the newly developing individual or ***embryo***. The egg usually is larger, therefore, than other cells in the animal which produced it. The fertilized egg (Exercise 12) is activated to divide repeatedly, by mitosis, into smaller and smaller cells that adhere to one another. The resulting cell mass is first solid and later hollow. Then the structural form and parts of the growing embryo are produced—by differences in rate of division of local cell groups, by outgrowths and ingrowths, by foldings, by cell migrations, and by other processes. Once started, embryonic development is continuous (some exceptions) and becomes progressively more complex. For convenience in study, however, the developmental history in any species is divided into a series of ***stages*** marking the more important events. In an introductory course, the structure of only a few conspicuous stages can be examined. To follow the many details of development requires a special course in embryology, even for the study of a few animal types.

The more conspicuous external changes in a developing egg or embryo can be seen in living material kept in the laboratory for some days (such as frog eggs); they also may be observed in a series of preserved specimens of the principal stages (text, Figs. 2·20, 5·9–13, 9·5, 13·6, 14·6, 19·8, 21·9, 23·11, 28·12, etc.). The many important internal and cellular changes, however, can be studied only in prepared specimens—stained whole mounts and stained microsections cut in one or more planes of the developing organism.

STARFISH

The egg and embryos of a starfish are small, have little yolk, and are transparent. By opening mature ("ripe") starfishes of both sexes, eggs and sperm may be obtained in numbers and mixed in sea water. Following such fertilization, various stages may be taken in ensuing hours, fixed, and mounted on slides.

1. Cleavage. The fertilized egg divides successively into 2, 4, 8, 16, . . . cells; this process is known as ***cleavage.*** Following each mitosis, the cytoplasm becomes divided by definite cleavage planes which show externally as furrows. Each component cell is termed a ***blastomere.***

Under high magnification of the compound microscope, examine a slide containing several entire eggs of a starfish (Asterias, etc.) in various stages of cleavage; find any or all of the following:

Unfertilized egg (single cell, enclosed only by cell membrane; contains one large nucleus with nucleolus)

Fertilized egg or zygote (single cell, surrounded also by fertilization membrane slightly outside cell membrane)

2-celled stage (of 2 blastomeres, each with nucleus; plane of cleavage passes in meridian [and "axis" of egg] from animal pole or hemisphere [with less yolk] to vegetal pole [with more yolk])

4-celled stage (of 4 blastomeres; second cleavage plane also meridional, but at right angle to first)

8-celled stage (8 blastomeres; third cleavage plane is equatorial, at right angles to both of preceding)

16-celled stage and later (cleavage planes more varied in direction)

Draw Fig. 1

The fertilization nucleus of the zygote (Exercise 12) contains chromosomes from both the male and female parents, and the cell divisions in embryonic development are by mitosis. What may be inferred, therefore, from a hereditary standpoint, as to the nuclear content of every blastomere?

2. Blastula. By repeated cleavages the blastomeres become progressively smaller and more numerous. The resulting cell mass first is solid and mulberry-like (morula); later the cells become arranged in a single-layered hollow sphere, the ***blastula,*** enclosing a cleavage cavity or ***blastocoel.*** *Under high magnification, examine a blastula; focus to obtain an "optical section" through the middle of the blastula showing the cell layer and blastocoel. Distinguish the animal and vegetal hemispheres by the size of their cells.*

Draw Fig. 2

When the starfish blastula consists of about 1,000 cells (how many "cell generations"?), it escapes from the fertilization membrane and can move about freely in the sea water by the action of cilia then present on the exterior of the blastomeres.

3. Gastrula. Mitosis thus far has been rather uniform, but local differences in division rate and growth now occur. The vegetal hemisphere invaginates or pushes into the blastocoel (eventually obliterating that cavity); as a result, the one-layered spherical blastula finally is converted into a two-layered cup or ***gastrula.*** This process is termed

gastrulation. The new cavity formed within the gastrula is the primitive gut or ***archenteron,*** and its external opening is the ***blastopore*** (future anus, at posterior end). The gastrula soon elongates on the body axis passing through the blastopore. The external cell layer is termed the ***ectoderm*** and that lining the archenteron is the ***endoderm;*** these are two of the germ layers from which organs and other parts later develop. Soon a third germ layer, the ***mesoderm,*** is produced by outpocketings (coelomic pouches) from the anterior end of the archenteron (text, Fig. 19·8*B*).

Study various stages showing invagination and gastrulation in optical section.

Draw Fig. 3

4. Later stages. Subsequent development in the starfish leads in turn to two stages of free-swimming larvae (bipinnaria and brachiolaria). The young starfish in time arises from part of a complex larval stage (text, Fig. 19·8). *If available, examine demonstration specimens of the larvae.*

FROG

The eggs of most frogs and other amphibians are laid in ponds or streams (text, pp. 40–41, 598–601), during the spring months. They may be taken in water to the laboratory where, with proper care, they will continue to develop, and the embryos later will hatch. At other seasons, frog eggs may be obtained by making pituitary implants into adult females to induce laying (ovulation); such eggs can be fertilized artificially by sperm taken from males, and then they will develop.

Eggs of amphibians are larger than those of starfishes, are colored dark by pigment over the animal hemisphere, and have much more yolk which is whitish and mainly in the vegetal hemisphere. The nucleus usually can be seen only in stained sections. Amphibian eggs and embryos are surrounded by gelatinous coatings (text, Fig. 29·6) which are added during passage through the oviducts and which swell soon after the eggs are laid in water. These must be removed from eggs that are to be prepared as whole mounts or sections.

Read Pars. 1 *to* 3 *on the starfish for description of some early processes in development; understand the meaning of the following terms:*

Egg axis	Cleavage	Gastrula	Germ layers
Animal hemisphere	Blastomere	Gastrulation	Ectoderm
Vegetal hemisphere	Blastula	Archenteron	Endoderm
	Blastocoel		Mesoderm

1. Cleavage. *Under low magnification with a hand lens or binocular microscope, study a series of frog* (or salamander) *eggs showing stages in cleavage; identify:*

Unfertilized egg (see also Exercise 1, Par. 3)
Fertilized egg or zygote (no fertilization membrane)
2-celled stage (of 2 blastomeres, each with nucleus)
4-celled stage

8-celled stage (4 smaller blastomeres [micromeres] and 4 larger [macromeres])
16-celled stage and later

From the 8-celled stage onward, the cells of the animal hemisphere are smaller; the large yolk-laden cells of the vegetal hemisphere divide more slowly and the number of blastomeres is usually irregular.

Draw Fig. 4

2. Blastula. After many cleavages and the production of great numbers of blastomeres, a ***blastula*** results in which the walls consist of several layers of cells (thus unlike the starfish). *Study a blastula in either* (*a*) *a stained vertical section, or* (*b*) *by examining the cut surface in a blastula bisected through both hemispheres; distinguish:*

Blastocoel (central cavity)
Animal hemisphere (cells small, with dark pigment granules)
Vegetal hemisphere (cells large and in a thick layer; some cell walls incomplete)
Yolk granules (minute, oval, within all cells)
Cell nuclei (seen in stained microscopic section)
Vitelline membrane (thin, noncellular, surrounds blastula; often broken)

Draw Fig. 5

3. Gastrula. The large amount of yolk in amphibian eggs prevents gastrulation by simple invagination (as occurs in the starfish). Instead, the small dark cells of the animal hemisphere grow down to cover gradually (epiboly) the large cells of the vegetal hemisphere, and there is also an inrolling (involution) of cells margining the blastopore. The end result, however, yields a gastrula (text, Fig. 5·10). In a late gastrula, some of the paler vegetal pole cells protrude near the margin of the blastopore as a ***yolk plug.*** Later this yolk plug is overgrown entirely and only the minute blastopore persists.

A. Study a median (sagittal) *section of frog gastrula in either* (1) *a stained and mounted microscopic preparation, or* (2) *by bisecting an entire gastrula through the blastopore and animal hemisphere to examine the cut surface; distinguish:*

Archenteron (interior cavity, the primitive gut)
Blastopore (near future anus, hence at posterior end; with dorsal and ventral lips, the latter toward vegetal hemisphere)
Yolk plug (of yolk-laden vegetal cells protruding near ventral lip of blastopore)
Ectoderm (cells small, in 2 or more layers, covering exterior of gastrula)
Endoderm (cells large and containing many yolk granules; in thick mass)
Mesoderm (thin layer of cells in roof of archenteron, beneath ectoderm)
Blastocoel (remains, if any)

Draw Fig. 6

B. *Similarly examine a cross section* (at mid-length) *of a late gastrula and identify such of the structures named in Par. A as can be seen.*

Draw Fig. 7

During gastrulation the axes and regions of the future body are established. The blastopore marks the posterior end, the opposite is anterior, and the longitudinal axis may therefore be visualized. The thick mass of endoderm cells from the vegetal hemisphere is ventral, and the thin layer of ectoderm cells (with mesoderm immediately beneath) is dorsal, over the open space of the archenteron. Mitotic figures now are common, especially in cells of the ectoderm and mesoderm.

4. The embryo. The late gastrula begins to elongate. From near the blastopore there develops forward in the dorsal ectoderm a shallow ***neural plate,*** having in the mid-line a furrow or ***neural groove*** and more laterally at either side a ***neural fold.*** These folds grow upward, converge, fuse dorsally to form the ***neural tube*** (forerunner of the central nervous system), and soon are covered over by the ectoderm. Anteriorly (region of future brain) the folds are widely separated until later in development. Meanwhile, immediately ventral to the neural plate, a median unsegmented rod of cells (mesoderm?) differentiates as the ***notochord,*** which serves as the axial support for the body until the vertebral column forms much later around the notochord. At either side of the notochord, the mesoderm grows downward laterally as a thin plate between the ectoderm and endoderm. Each plate of mesoderm soon splits into two layers: the outer (parietal), together with the ectoderm, forms the body wall; and the inner (splanchnic) layer surrounds the endoderm of the digestive tract to produce the outer parts of that tract (Exercise 1) and the mesenteries and peritoneum. The space between the two layers increases to become the body cavity or ***coelom.***

5. Neural groove stage. *Examine an entire embryo of this stage to see the external features; then study a stained cross section and identify:*

Neural plate (cells tall)
Neural groove (1; middorsal)
Neural folds (2; dorso-lateral)
Notochord
Archenteron
Coelom (beginning of)
Ectoderm (thin; cells small)
Endoderm (thick mass; cells contain much yolk)
Mesoderm
  Parietal layer (against ectoderm)
  Splanchnic layer (against endoderm)

Draw Fig. 8

6. Neural tube stage. *Study a stained cross section of an embryo in which the neural tube has formed.* The tall cells (earlier seen in the neural plate) are arranged radially around the central canal of the neural tube, which is higher than wide. *Identify the structures listed under Par.* 5.

At either side of the neural tube and notochord is a thick mass of mesoderm (segmental mesoderm), which later will contribute to formation of the dermis (deeper layer) of the skin, the segmental muscles of the back, and the early excretory system.

Draw Fig. 9

7. Later embryos. *Examine older entire embryos of one or more stages between that of the neural tube and hatching. Notice the progressive changes in form and size. Identify such of the following features as are present in each stage:*

HEAD

Eyes (2; lateral, indistinct)
Olfactory pits (2; anterior)
Stomodeum or mouth pit (median, antero-ventral; later connects to digestive tract)
Oral suckers (2; ventral; of horseshoe shape; used after hatching for clinging to objects in water)
Gills (2 pairs; lateral, on "neck" region; later 3 pairs, branched and plume-like)

TRUNK

Dorsal ridge (along back, with beginning of median fin)
Somites or myotomes (forerunners of segmental muscles, below dorsal ridge, separated by shallow < -shaped furrows)
Ventral mass (swollen, over yolk-laden endoderm)
Anus (minute, at base of tail)

TAIL

Median fins (dorsal and ventral)
Myotomes (lateral)

8. Larval stages. When the embryo has attained a certain stage in development, it hatches or escapes from the gelatinous egg coverings (text, Fig. 2·20) and is free to swim in the water. The individual then is called a ***larva.*** The reserve of yolk is soon exhausted; the larva then takes food, grows, and develops further. The length of the larva at hatching and the duration of life as a larva vary among different species of frogs and salamanders (text, Chap. 29).

The frog larva (tadpole or pollywog) has a combined head and trunk of ovoid form and a long tail with median dorsal and ventral fins. The external gills present at hatching are soon replaced by internal gills (text, Fig. 29·4), and a thin membrane, the ***operculum,*** grows over the gill apertures and body. The ***limbs*** appear late in larval life. ***Metamorphosis,*** the transformation from larval to adult form, requires some days for completion. It involves conspicuous changes that include widening of the mouth, resorption of the gills, beginning of lung respiration, complete resorption of the tail and its fins, and shortening of the intestine. The alterations in the mouth and intestine are related to a complete

change in food: the larva feeds upon minute vegetable materials (algae, etc.), whereas the transformed frog subsists upon entire insects and other small animals.

Examine a series of larval specimens in various stages of growth and during metamorphosis, and study the changes in external features.

BIRD

The eggs of reptiles and birds contain large amounts of yolk which serve to nourish the embryo during its development. These eggs are not deposited in water, and protection against drying during embryonic development is afforded by coverings consisting of albumen ("egg white"), shell membranes, and a shell. Cleavage and gastrulation in bird eggs occur before they are laid and in a somewhat different manner (text, Fig. 5·9*C*) from that in the frog.

To obtain embryos for study, the eggs are incubated (the age of a bird embryo is stated in hours of incubation). Then a hole is cut in the shell and the egg is immersed in warm salt solution (0.9 per cent NaCl). The yolk always revolves so as to bring the embryonic area (blastodisc) uppermost. This area is cut free with fine scissors, floated off and spread in fixing fluid, then washed and stained; finally it is prepared either as a whole mount to show general structure or as serial sections for study of the internal details.

1. Unincubated egg. *Examine an unincubated hen's egg that has been opened, and identify:*

Shell	Shell membranes (2; close together, lining inside of shell)
Albumen	Chalazeae (2 twisted cords of albumen at ends)
Yolk	Blastodisc (whitish embryonic area on yolk)

2. Chick embryo. *Examine demonstrations of any or all of the following stages of chick embryos to see the progressive development of structural parts* (text, Figs. 5·12, 5·14), *and identify those named:*

16 to 20 hour stage (primitive streak, notochord, neural plate)
24 hour stage (head fold, neural folds, notochord, mesodermal somites; area pellucida [clear, around embryo]; vascular area [outside preceding])
36 to 43 hour stage (brain, eye vesicles, heart, veins, anterior fold of amnion)
48 hour stage (ear vesicles, aortic arches, and gill slits; neural tube; additional somites; extension of amnion)
72 hour stage (buds of future wings, legs, and tail; additional somites; further growth of amnion; extensive development of arteries and veins over surface of yolk)

DRAWINGS

STARFISH

Make each figure 30 *mm. or more in diameter; show and label all parts identified.*

Fig. 1. 1-, 2-, 4-, 8-, and 16-celled stages, in outline.

Fig. 2. Blastula in optical section.

Fig. 3. Gastrula, lengthwise optical section (with blastopore at one end).

Frog

If directed to do so, color germ layers in Figs. 6–9 lightly, as follows: ectoderm, *blue;* endoderm, *yellow;* mesoderm, *red;* nervous tissue, *green;* notochord, *brown.*

Fig. 4. 1-, 2-, 4-, 8-, and 16-celled stages, in outline (each 30 mm. in diameter); show cleavage furrows; label animal and vegetal hemispheres on one.

Fig. 5. Blastula (40 mm. in diameter), median section; show also one cell enlarged with nucleus and yolk granules.

Fig. 6. Gastrula (50 mm. in diameter), sagittal section; outline layers of cells and show a few of each layer in detail.

Fig. 7. Gastrula (50 mm. in diameter), cross section, same style as Fig. 6.

Fig. 8. Neural groove stage (50 mm. high), cross section. Outline parts named in Par. 5 and show a few cells of each in detail.

Fig. 9. Neural tube stage (50 mm. high), cross section. Same style as Fig. 8.

EXERCISE 14. HEREDITY AND GENETICS

(Storer, "General Zoology," pp. 140–147, 154–156, 158–159)

This exercise will serve primarily to show (1) the basic phenomena of heredity by rearing two generations of the fruit fly (*Drosophila melanogaster*) in which the inheritance of some simple characters will be followed; it also will illustrate (2) the life cycle of an insect with complete metamorphosis (text, p. 482), and (3) the geometrical ratio of increase in a population (text, pp. 127, 218).

Drosophila is used commonly for laboratory studies in genetics (the science dealing with heredity) because (1) it is small and is easily reared in numbers; (2) its complete life cycle is short, 10 to 14 days at ordinary temperatures; and (3) many stocks are available that have distinct hereditary differences (mutant characters) in color or structure of the eyes, wings, body, and other parts.

Fruit flies ordinarily lay their eggs on decaying fruit where the larvae (and adults) find abundant food in the wild yeasts growing on such fruits. Laboratory stocks of these flies (free of molds that would contaminate and interfere with experiments) are reared in plugged milk bottles containing banana pulp, or corn meal and molasses, or a chemical mixture, that serves for growth of pure yeast "seeded" into the bottles. Agar from seaweed is added to hold the culture materials as a firm mass when the bottle is handled, and rough towel paper is placed in each bottle to receive the eggs and pupae. To avoid mold contamination, the bottles and the culture medium are sterilized with heat. Later, when cooled, pairs of flies are introduced. Care is used to handle the flies with clean instruments and to keep the bottle stoppers from touching tables, etc., to avoid mold spores from the laboratory entering the bottles. The bottles usually are kept in an incubator at about 25°C. to ensure uniform growth of the larvae.

BIOLOGY OF DROSOPHILA

1. Adult flies. *Obtain some etherized adults, place them in a watch glass or on a slide, and examine under low magnification to learn their characteristics:*

BOTH SEXES. Head with short antennae and compound eyes; thorax with 3 pairs of legs and 1 pair of wings (Order DIPTERA) that, when folded normally, extend beyond the tip of the abdomen; abdomen with distinct somites and cross-banded with black.

MALE. Slightly smaller; abdomen parallel-sided, with 3 black crossbands, the last extending beneath the rounded and blunt posterior end.

FEMALE. Abdomen broader and swollen, with 5 crossbands; end pointed and protruding, no black beneath.

Flies etherized until dead have the wings up at an angle with the body; recently emerged flies are pale and the abdomen of young females is small.

Draw Fig. 1

2. Stages in life cycle. *A. Under low magnification, examine each of the following:*

EGG. Minute, oval, white, with 2 oar-like projections that prevent the egg from sinking into pulpy materials.

LARVA. Hatches from egg as small slender white worm or "grub"; body segmented, without distinct head or thorax; no true legs; anterior end with 2 black chitinous jaws; posterior end with 2 dorsal spiracles connected to longitudinal tracheal ducts of the respiratory system that carry air; size of larva increases at each of the several molts of the body covering.

PUPA. After attaining its growth, the larva attaches to some object and the last larval skin becomes a semitransparent puparium (later darkened) within which the larva transforms or metamorphoses into a fly. *In an older pupa, distinguish:*

Pupal spiracles (2; horn-like, dorsal near anterior end)
Compound eyes (ventral, behind anterior end of puparium)
Wings (ventral, folded)
Legs (extended backward between wings)

Examine an empty pupa case from which a fly has emerged.

Draw Fig. 2

B. Examine from day to day a culture bottle into which one or more pairs of Drosophila were introduced, and keep record of the appearance of eggs, larvae, and pupae, and of the emergence of adult flies.

How many days were required for each stage? For the complete life cycle? Would change in the environmental temperature affect the time,

and how? How many flies were produced? What is the proportional increase? What is meant by geometrical ratio of increase? If all offspring survived and produced at the same rate, what would the population (starting from 1 pair) be in 100 days? What factors in nature are operating to prevent such a great increase? Does a fly "grow" after hatching from its pupal case?

GENETICS OF DROSOPHILA

3. Terminology. *Learn the meaning of the following terms which are used commonly in discussing problems of inheritance* (see text, Chap. 6 and Glossary):

P_1	Dominant	Character	Genotype	Homozygous	Segregation
F_1	Recessive	Allele	Phenotype	Heterozygous	Autosomal
F_2	Hybrid	Gene (factor)	Mutation		Sex-linked

Who was Mendel? What was his important contribution to the study of inheritance? What are his two laws? What is a monohybrid cross?

4. Experimental crosses. *Each student (or pair of students) should make two crosses: one with some autosomal character such as normal wings vs. curly wings, etc., and another with a sex-linked character such as red eye (normal) vs. white eye. At each laboratory table one or more matings should be normal female vs. curly male and others of the opposite or* ***reciprocal cross,*** *normal male vs. curly female. In the sex-linked experiment, reciprocal matings also should be made: red-eyed female vs. white-eyed male, and red-eyed male vs. white-eyed female.*

The instructor will indicate the dates when the P_1 *flies are to be released, the* F_1 *offspring counted and* F_1 *matings made, the* F_1 *flies released, and the* F_2 *offspring counted.*

Begin Table 1 *when the first matings are made, and complete the entries and calculations after all* F_2 *flies have been counted.*

Prepare Table 1

5. Laboratory procedures. Mold-free stocks of homozygous flies are maintained to provide for experimental crosses. To ensure that the females used are unmated (virgin), they must be removed from the stock culture bottles within a few hours after emerging from the pupal cases.

Whenever a culture bottle is opened, the cotton plug should be grasped between the backs of two fingers and held free of contact with the table or other objects to avoid contamination with mold spores.

A. MAKING THE CROSSES. A culture bottle and one or two pairs of freshly emerged parent flies (P_1) will be provided for each cross. *Label each bottle with the necessary data, as follows:*

Student's name	JOHN BROWN
Laboratory section and seat	I-18
Date cross is started	10-16-41
Generation	P_1
Symbols for parents	♂ *NN* − ♀ *nn* (or $X_RX_R - X_rY$)

(Later when the F_1 *matings are made, the bottle for each cross should bear the same symbols as used for the parent mating, to avoid confusion in recording the results.)*

To place the parent flies in the bottle, remove the stopper, lay the bottle on its side, use a narrow strip of clean paper to introduce the etherized flies, and replace the stopper. After the flies recover from the anesthetic and move about freely, the bottle may be stood upright and then placed in the incubator.

Examine the culture bottles at each subsequent laboratory period. If mold appears, or if the flies are lost, or if larvae fail to appear in numbers by the seventh day, report to the instructor for replacement materials or cultures.

On the tenth day remove the flies (P_1 or F_1) *used for matings so that these individuals will not mate with their offspring and complicate the results.*

B. TRANSFER OF FLIES. *On the days appointed for counting the* F_1 *and* F_2 *flies, respectively, handle the bottles as follows. The flies are positively phototropic, tending to go toward a light. Hold the culture bottle with its stoppered end away from the light, then the flies will move toward the culture medium. Covering the upper part of the bottle with the hand or a black cloth may help, as will giving the bottom of the bottle a sudden thrust against the opposite hand. When the flies are concentrated toward the bottom, quickly remove the stopper and bring the mouth of an empty etherizing bottle against that of the culture bottle. While keeping the two in contact, reverse their position so that the empty bottle is toward the light. Gently tapping the base of the culture bottle may encourage the flies to move. As soon as they have passed into the second bottle, stopper the latter with a cork holding cotton moistened with ether and close the culture bottle. Two students may work to advantage here, one holding the etherized stopper ready while the second is transferring the flies.*

C. ETHERIZING. *If some flies are needed for mating* (in the F_1 generation), *allow the ether to act for only about* 20 *seconds after the flies have ceased to move; then empty them onto a clean sheet of white paper, the edges of which have previously been turned up about* ½ *inch all around to prevent loss of flies. Select those wanted for mating and place in a new culture bottle, properly labeled; then return the remainder of the flies to the etherizing bottle for* 2 *minutes or more until all are killed.*

D. COUNTING. *Turn the flies out on paper and use a soft brush or forceps to divide them into groups, by characters and by sex; count the*

individuals in each, and record (including also any removed for other matings). *Flies from each culture bottle must be counted separately.*

In the autosomal cross, which is the dominant character? Does the recessive character appear in any F_1 offspring? What proportion of the F_2 flies show the dominant character? Are these all homozygous? Theoretically what ratio of phenotypes should be obtained in the F_2? Were the actual results in the same ratio? What bearing do meiosis and fertilization have on the characteristics appearing in hybrid offspring? On the ratios between dominant and recessive phenotypes obtained? Are the results influenced by the sex of the dominant parent? Why?

What are some examples of Mendelian characteristics in other animals?

In the sex-linked cross, what effect does sex have on the characteristics appearing in the offspring? On the ratios of these characteristics? Are the results different from those in the autosomal cross? Why?

What was the actual sex ratio of offspring from each mating? Theoretically what should be the sex ratio in each?

What are some examples of sex-linked characteristics in mankind?

What are some practical results in genetics?

DRAWINGS

Fig. 1. Abdomen of *Drosophila*, male and female, ventral view (×5).

Fig. 2. Egg, larva, and pupa of *Drosophila* (×10).

TABLE 1.—REPORT ON MONOHYBRID CROSSES IN DROSOPHILA*

AUTOSOMAL CROSS

(*a*) Normal ♂ (*NN*) and ..... ♀ (*nn*)

(*b*) ..... ♂ (*nn*) and normal ♀ (*NN*)

SEX-LINKED CROSS

(*c*) Red-eyed ♀ (X_RX_R) and white-eyed ♂ (X_rY)

(*d*) White-eyed ♀ (X_rX_r) and red-eyed ♂ (X_RY)

		Males		*Females*	
P_1 Parents	Phenotypes				
	Genotypes				
	Gametes				
F_1 Generation	Genotypes, possible				
	Phenotypes, possible				
	Phenotypes, actual counts				
	Phenotypes, ratios				
	Actual counts of sexes				
	Sex ratio, expected				
	Sex ratio obtained				
	Gametes				
F_2 Generation	Genotypes, possible				
	Phenotypes, possible				
	Phenotypes, actual counts				
	Phenotypes, ratios				
	Actual counts of sexes				
	Sex ratio, expected				
	Sex ratio obtained				

* Indicate which crosses were made; write name of character used in (*a*) or (*b*); enter symbols or numbers as necessary to complete the table; calculate sex ratios as males per 100 females.

EXERCISE 15. ECOLOGY AND DISTRIBUTION

(Storer, "General Zoology," pp. 167–196)

Ecology is the subdivision of biology that deals with living organisms in their natural environments and that seeks to determine the factors responsible for their presence or absence in any particular place. ***Distribution*** deals with the occurrence of animals over the earth and the environments that each utilizes (text, p. 182, etc.). An introductory course in zoology touches various aspects of animal life, including ecology and distribution, but for practical reasons the laboratory exercises must be confined mainly to the study of structure with some attention to function. It is difficult and expensive of time and materials to rear and keep many kinds of living animal under conditions such that their normal habits may be observed. On the other hand, the study of animals in their natural environments requires more time than is available during an elementary course.

A short field trip, however, will provide brief acquaintance with some species and of the relations that they bear to their surroundings and to one another. The conditions for such a trip will vary with locality and require special directions by the instructor; hence no details of procedure are outlined. Attention should be given to the topics mentioned here.

A field trip may include one or more of the following kinds of environment:

Seashore (rocky, sandy, muddy)
Salt- or fresh-water marsh
Fresh-water river, stream, pond, or lake
Alkaline lake or stream
Grassland (various sorts)
Brushy or shrubby area
Forest (dense or scattered, coniferous, deciduous, or mixed)
Rocky area or outcrop
Sandy or alluvial land

A locality affording more than one of these kinds of environment is preferable. Why?

Whatever the locality, the student should analyze the subdivisions of the environment and determine which of them are inhabited by animals—large, small, or microscopic. Also he should seek to determine why each has particular advantages for one or another type of animal.

The problem of an individual animal is to survive successfully, at least until it has reproduced. The problem of a species is to maintain such numbers, even under the most unfavorable conditions, that its continuance will be possible. The requirements of any animal (including man) may be resolved into major factors that include

Food Shelter Breeding places Competition Enemies Disease

Careful analysis of these, even in the case of a single species, can be made only by long and intensive study, but brief observations will sug-

gest the importance of some factors as they affect certain types of animals.

If time and laboratory facilities permit, some kinds of animals (especially small aquatic species) may be collected and taken to the laboratory for further study.

EXERCISE 16. ADAPTATION

(Storer, "General Zoology," pp. 178–180; 200–201; 238–240; 471–476)

A ***characteristic*** is any feature or peculiarity of an organism or group of organisms with respect to structure, function, or behavior. Any characteristic that is modified from the general type and enables an animal to do the things necessary for its particular existence more efficiently or more successfully is termed an ***adaptive characteristic*** or an ***adaptation.*** Some adaptations are individual or acquired, and others are racial or inherited. Examples of acquired characteristics in mankind are the enlargement of muscles by continued exercise (structure), the tanning of the skin following exposure to strong sunlight (function), and the acquisition of skill in particular kinds of work or games (behavior). Inherited adaptive characteristics are those which enable all individuals of a species or larger animal group to live successfully in some special environment, or in a particular manner, or to do both of these. The wings of birds, bats, and insects, which enable these animals to travel about readily in the air, are inherited adaptive characteristics. Hereditary adaptations are of particular importance in the study of evolution, which seeks to account for their origin and development. The evolution of any adaptation is believed to have been from a generalized condition to one of greater specialization. Thus, in land vertebrates (amphibians to mammals) the acquisition of two pairs of typically 5-toed limbs preceded the development of specialized types of limbs and toes among various groups and species (text, Fig. 9·2).

1. Insect appendages. The crustaceans, insects, spiders, etc. (Phylum ARTHROPODA), all possess pairs of jointed appendages on their bodies. These include legs for walking (swimming in aquatic types) and other appendages that serve in feeding, offense and defense, reproduction, and other essential activities. (Details of modifications in appendages of the crayfish are dealt with in Exercise 38.)

Among insects (Class INSECTA), except for some legless types, the many species all have three pairs of legs, constructed on a common plan. Then among the various orders, families, and species, the legs often are adaptively modified for particular modes of life. As examples, those of the grasshopper are rather generalized (although specialized for leaping), whereas in the honeybee they are highly specialized for collecting pollen from plants (text, pp. 471–476; Figs. 23·13, 23·14).

A. COMMON CHARACTERISTICS. *Examine the structure of the three legs on one side of the body in both of these insects and identify the following components in each leg:*

Coxa (short, jointed to body)
Trochanter (short)
Femur (large)
Tibia (slender)
Tarsus (terminal, variously divided)

Study the use of the legs in living examples of these two or any other species of insects that may be available. Are the legs used in essentially the same way for walking by all insects?

B. ADAPTIVE MODIFICATIONS. *Study now the detailed differences between the three legs on one side, first in the grasshopper and then in the honeybee.*

The hind legs of the grasshopper assist in walking and also are the means of jumping. How are the component segments adapted for the latter purpose in respect to size, length, and other features (structure)? Is the normal "resting" position of the hind legs adapted for quick action in leaping (function)? How does the position compare with the hind legs in a resting frog? What does either of these animals do when suddenly disturbed (behavior)?

In the honeybee identify the following adaptive characteristics and learn how each is used:

FORELEG	MIDDLE LEG	HIND LEG
Eye brush	Pollen brush	Pollen basket
Velum	Spur	Pecten
Antenna cleaner		Auricle
Pollen brush		Pollen brush
		Pollen comb

Of what adaptive significance is the general hairy covering of the honeybee as compared with the smooth body of the grasshopper?

2. Insect wings. *A. Compare the spread wings of the grasshopper and honeybee.* The leathery forewings of the grasshopper act as protective covers for the folded thin membranous hind wings when the animal is not in flight. In the honeybee both pairs of wings are membranous, and during flight those on each side are fastened together by hooks and grooves.

From the standpoint of life on the ground and amid grasses and plants, of what adaptive advantage are the leathery forewings of the grasshopper? What would happen if both pairs of wings were delicate? What advantage accrues from the folding of the hind wings when at rest? In respect to habits, why are such adaptations unnecessary in the honeybee?

B. Examine the types of wings in various orders of insects, either in demonstration specimens or in illustrations (text, Figs. 23·19–23·47). *Note first the common characteristics such as number of wings, their general structure, and presence of supporting "veins." Then endeavor to determine which characteristics in each of several kinds of insect are adaptive.* Is the

absence of wings in fleas, certain castes of ants and termites, and some other insects to be considered adaptive? If so, why?

3. Vertebrate appendages. ***A.*** COMMON CHARACTERISTICS. *Compare demonstration specimens of skeletons or illustrations to determine the common features of the paired limbs in land vertebrates. See text* (Table 4·1) *and such types as the following:*

Frog (Fig. 2·9)
Man (Fig. 4·5*A*)
Crocodile (Fig. 30·3)
Bird (Figs. 31·5, 31·6)
Cat (Fig. 32·2)
Other types (Figs. 9·1, 9·2)

B. ADAPTIVE MODIFICATIONS. *Study the ways in which the limbs of several types of vertebrates are adapted for the particular mode of life of each. Notice the relative size and length of the component bones, differences in their shape, and the fusion or loss of certain bones.*

Are the limbs of mankind generalized or specialized, and why? What are some adaptive modifications in the limbs of horses, cattle, deer, and similar types that suit them to rapid travel in open country? What sorts of modifications in the limbs of bats and birds, respectively, adapt these animals for flight? For what specialized mode of life is the loss of limbs in snakes adaptive? Did the latter animals probably derive from ancestors with limbs (text, p. 619; Fig. 30·9)?

What is the distinction between homology and analogy in respect to animal characteristics (text, pp. 200–201, 239, Fig. 9·1)?

4. Bills of birds. *Compare the bills of several kinds of birds for adaptive modifications to particular methods of obtaining food* (text, Fig. 31·11), *including such kinds as*

Duck (broad, edges with thin parallel ridges to sieve minute food from water)
Snipe or other "shore bird" (slender, long, for probing in mud or sand)
Woodpecker (slender, rigid, for cutting and probing in wood)
Hawk or owl (short, stout, sharp-edged for cutting and tearing flesh)
Sparrow (conical, for crushing seeds)
Flycatcher or poorwill (delicate, broad, for capturing flying insects)

The snipe and woodpecker, or the hawk and owl, are examples among birds belonging to different ancestral groups (orders) that have bills of somewhat similar form and purpose—examples of ***parallel adaptation.***

5. Teeth of mammals. *Examine the teeth of some different types of mammals* (text, Fig. 32·11) *that are adapted by structure to deal efficiently with different types of food, such as*

ADAPTIVE TYPE	EXAMPLES
Insect-eating (insectivorous)	Mole, shrew, bat
Flesh-cutting (carnivorous)	Dog, cat, seal, bear
Cutting off and grinding vegetation (herbivorous)	Horse, cow, or deer; also beaver, muskrat, or other rodent

EXERCISE 17. CLASSIFICATION

(Storer, "General Zoology," pp. 238–254)

Upwards of 900,000 kinds of living animals are known already, and others are being discovered and described. It is the purpose of zoological ***classification*** (known also as taxonomy or systematic zoology) to group and arrange these many animals for study. Classification serves (1) to identify individual specimens, (2) to provide means to comprehend the number and variety of animals, (3) to guide the arrangement of specimens in scientific collections of animals, and most importantly (4) to express the relationships between the various kinds and larger groups as a means of understanding their probable manner of origin (organic evolution, text, Chap. 9).

Classification is based upon the characteristics or inherent peculiarities of the animals as to structure, mode of development, and other features. The Animal Kingdom is divided first into major groups or phyla. All animals in each phylum have certain common characteristics. Then each phylum is divided successively into smaller and smaller groups: classes, orders, families, genera, and species (Fig. 11). The basic unit in classification is the ***species*** (text, p. 239).

The beginning student may obtain a perspective of the size and diversity of the Animal Kingdom by classifying some representative animals to the phylum, class, and order in which each belongs. Such experience is of value in showing the methods and the kinds of characteristics employed in classification and affords some familiarity with the larger subdivisions, the names of which are used frequently. It is not practicable to extend such an exercise down to the smaller groups because the keys and descriptions needed to classify specimens to species are available only in special books and other publications. Such work, however, is done in some advanced courses in zoology.

1. Classification. *Using a numbered series of representative specimens, study each in turn; determine its more general characteristics by which it may classified as belonging to a particular phylum. Then place each in its appropriate class (and order where possible).* Use text Table 11·1, the "synopsis of classification" (text, pp. 247–254) and the "key" at the end of this exercise. *After the position of a specimen has been determined, refer to the more detailed summaries of classification* (at ends of text Chaps. 12–32) *and compare the specimen with any text figures available to aid in confirming the identification.*

2. Species, genera, etc. *If available, examine a demonstration series of specimens from some one of the larger groups* (mammals, birds, insects, mollusks, etc.) *chosen to illustrate the smaller categories in classification:*

Species (several examples to illustrate individual variation)
Genus (several different species to show the characteristics that separate species from one another)
Family (examples of several genera to show the common characteristics by which they all are placed in one family and also the characteristics by which the genera are separated from one another)

TABLE 1.—LIST OF REPRESENTATIVE ANIMALS CLASSIFIED

No.	Phylum	Class	Order	Common name
1				
2				
etc.				

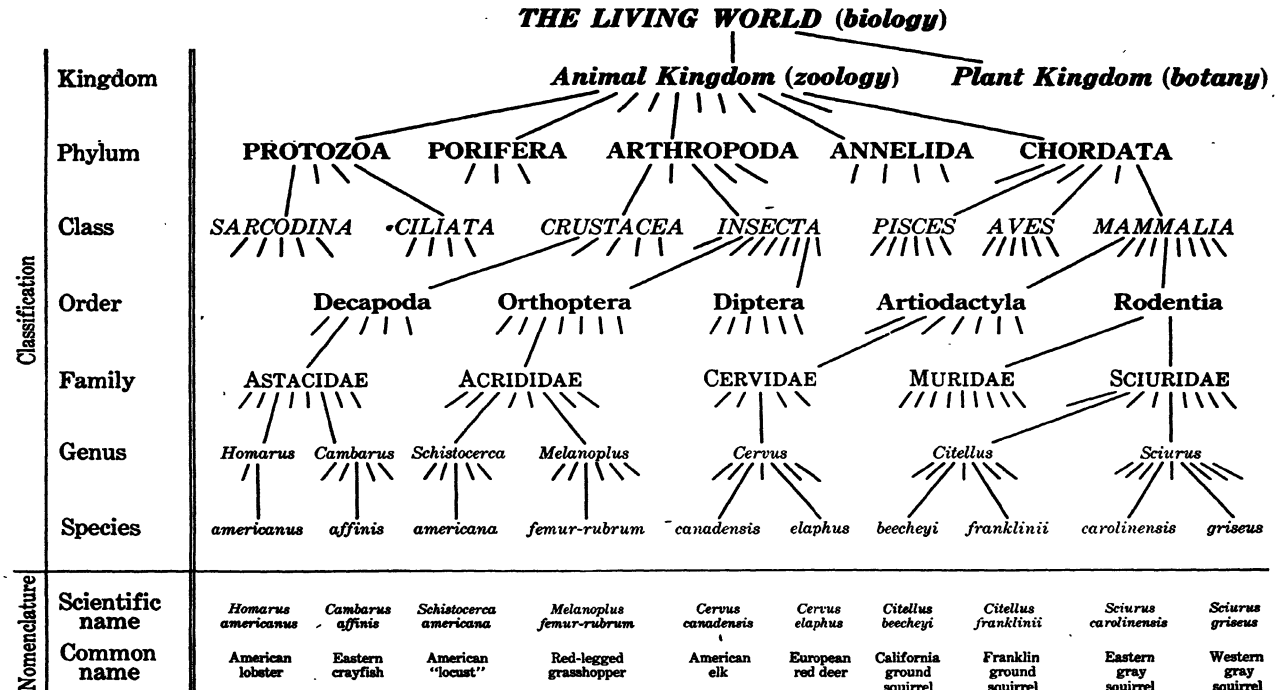


FIG. 11.—Diagram to indicate how animals are classified. The Animal Kingdom is divided into about 20 phyla (only 5 of which are included here for lack of space). Each phylum in turn is subdivided into several classes (only 3 of 15 classes are here named under Phylum CHORDATA), each class into orders, and so on down to species. Long lines indicate examples carried entirely or partly through in classification; short lines indicate that other subdivisions of groups have been omitted.

The scientific name, composed of the genus and species names, is that by which an animal species is known to biologists in all countries. The common name may be used only locally (text, pp. 244–245).

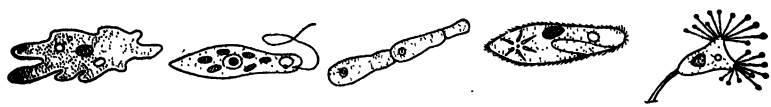
KEY TO THE ANIMAL KINGDOM

This key will serve to identify many animals to phylum and class (a few groups are omitted). Each statement is preceded by a number and another in parentheses. If the specimen agrees with a statement, then the next statement in numerical sequence should be read; but at any place where it does not agree, the alternative statement (of the number in parentheses) should be consulted; continue until the specimen has been identified. To verify, consult the summaries of classification in Storer, "General Zoology," Chaps. 11 and 12 to 32.

For example, the correct identification of a frog as a member of the Phylum CHORDATA and Class AMPHIBIA would trace through Nos. 10, 16, 25, 82, 88, 90, 94, 95.

Names of phyla are in boldface capitals (Ph. **PROTOZOA**) and of classes in italic capitals (Cl. *SARCODINA*). See Glossary (text, pp. 743–758) for definitions of special terms as necessary.

1(10) Animals of one cell, or in colonies of like cells (rarely a mass of protoplasm containing many nuclei); no tissues; size usually microscopic..... .....UNICELLULAR ANIMALS—Subkingdom PROTOZOA Ph. **PROTOZOA**



SARCODINA MASTIGOPHORA SPOROZOA CILIATA SUCTORIA

FIG. 12.—Phylum PROTOZOA. Representatives of the 5 Classes.

2 (7) No cilia.....Subphylum PLASMODROMA
3 (6) With special cell parts (organelles) for locomotion
4 (5) Pseudopodia for locomotion.....Cl. *SARCODINA*
5 (4) Flagella (1 to many) for locomotion...FLAGELLATES Cl. *MASTIGOPHORA*
6 (3) No organelles for locomotion (except in some young); all parasitic, producing spores.....Cl. *SPOROZOA*
7 (2) With many hair-like cilia for locomotion.....Subphylum CILIOPHORA
8 (9) Cilia always present.....CILIATES Cl. *CILIATA*
9 (8) Cilia only in young; later with "tentacles".....Cl. *SUCTORIA*
10 (1) Body of many cells, of different kinds, and arranged as layers or tissues..... .....MULTICELLULAR ANIMALS—Subkingdom METAZOA
11(16) Body wall pierced by many pores, and a large osculum (or several); exterior often rough or bristly, symmetry partly radial or none, shape various, often irregular or branched; no digestive tract or other organs; skeleton of spicules or fibers (text, Fig. 13·5).....SPONGES Ph. **PORIFERA**
12(13) Spicules calcareous, body and osculum often bristly.....Cl. *CALCAREA*
13(12) Spicules not calcareous
14(15) Spicules siliceous, 6-rayed.....Cl. *HEXACTINELLIDA*
15(14) Spicules and skeleton various, often with spongin...Cl. *DEMOSPONGIAE*

PORIFERA HYDROZOA SCYPHOZOA ANTHOZOA CTENOPHORA

FIG. 13.—Phyla PORIFERA, COELENTERATA (3 Classes), and CTENOPHORA.

16(11) Body not pierced by pores, and of some regular form; mouth and digestive cavity or tract present (except Nos. 33, 39)

17(25) Symmetry radial or biradial around a central oral-aboral axis; no "head" or "tail"

18(47) Parts in 4's or 6's (if in 5's, see No. 47); body soft, no internal skeleton

19(24) Mouth or oral surface with soft unbranched tentacles that bear microscopic nematocysts; no ciliated comb plates.....Ph. **COELENTERATA**

20(23) No gullet inside mouth; no septa dividing digestive cavity

21(22) Either cylindrical polyps (often in colonies) with mouth on hypostome, or gelatinous medusae with velum.....HYDROIDS Cl. *HYDROZOA*

22(21) All free-floating gelatinous medusae, the body bell- or umbrella-shaped, and notched at margin; oral arms about mouth; no velum.....JELLYFISHES Cl. *SCYPHOZOA*

23(20) A gullet inside mouth and septa dividing digestive cavity; sessile; no body stalk; all sessile polyps.....SEA ANEMONES AND CORALS Cl. *ANTHOZOA*

24(19) Tentacles not around mouth; 8 meridional rows of ciliated comb plates; no nematocysts.....COMB JELLIES Ph. **CTENOPHORA**

25(17) Symmetry definitely bilateral, in at least part of body (some with parts of spiral or U-shape)

26(82) No gill slits on pharynx and no internal skeleton (skull, vertebrae) of bone or cartilage

27(40) Body variously slender and worm-like, but without lateral appendages, fins, true segments (somites), or shell

28(35) Body soft, flat (rarely cylindrical)

29(34) Body flattened dorso-ventrally, often leaf-like (if long, with pseudosegments); no anus or proboscis; sexes united (monoecious).....FLATWORMS Ph. **PLATYHELMINTHES**

30(33) Mouth and digestive tract present; no pseudosegments

31(32) No suckers about mouth; epithelium ciliated; no cuticle.....Cl. *TURBELLARIA*

32(31) Suckers about mouth or elsewhere; some with posterior hooks; shape leaf-like or slender; minute to 75 mm. long; parasitic....FLUKES Cl. *TREMATODA*

33(30) No mouth or digestive tract; body of a scolex with suckers and few to many similar pseudosegments (proglottids) of progressively increasing size; parasitic.....TAPEWORMS Cl. *CESTOIDEA*

34(29) Body long, slender, soft, highly contractile; often brightly colored; ciliated; a protrusible proboscis above mouth; anus present; mostly free-living.....RIBBON WORMS Ph. **NEMERTINEA**

35(28) Body narrowly cylindrical; cuticle thick

FIG. 14.—Phyla PLATYHELMINTHES (3 Classes), NEMERTINEA, and NEMATHELMINTHES.

36(37) Lateral lines present.....ROUNDWORMS Ph. **NEMATHELMINTHES**

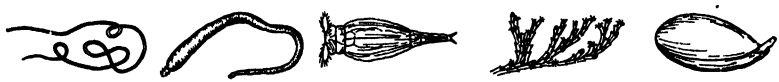
37(36) No lateral lines

38(39) No proboscis; body extremely slender, blunt at ends.....HORSEHAIR WORMS Ph. **GORDIACEA**

39(38) A spiny retractile proboscis; parasitic.....SPINY-HEADED WORMS Ph. **ACANTHOCEPHALA**

40(27) Body shape various; if worm-like, has appendages or is segmented, or both

41(42) Body slender with 2 or 3 pairs of lateral fins; bristles at mouth; no cilia; length to 70 mm.....ARROW WORMS Ph. **CHAETOGNATHA**



GORDIACEA ACANTHOCEPHALA TROCHELMINTHES BRYOZOA BRACHIOPODA

FIG. 15.—Miscellaneous phyla.

42(41) No lateral fins
43(46) Individuals minute or microscopic, with cilia; no segmentation
44(45) Anterior end with discs of cilia; jaws internal..... .....ROTIFERS Ph. **TROCHELMINTHES** Cl. *ROTIFERA*
45(44) Anterior end with crown of ciliated tentacles, often retractile; no jaws; colonial, sessile.....MOSS ANIMALS Ph. **BRYOZOA**
46(43) Individuals variously sized and shaped, some segmented (proceed to No. 56 next)
47(18) Body parts in 5's (sometimes obscure); symmetry radial; body wall hard, spiny, or leathery; usually with delicate slender soft tube feet; tentacles branched, if present.....ECHINODERMS Ph. **ECHINODERMATA**

ASTEROIDEA OPHIUROIDEA ECHINOIDEA CRINOIDEA HOLOTHURIOIDEA

FIG. 16.—Phylum ECHINODERMATA. The 5 living Classes.

48(55) Body not elongate; skeleton of hard plates or ossicles; no branched tentacles
49(50) Body calyx-like; both mouth and anus on upper surface; usually with stalk and commonly attached; arms slender, branched..... .....SEA LILIES Cl. *CRINOIDEA*
50(49) Body various (not flower-like); animal moves on oral surface
51(54) Arms present
52(53) Body star-shaped or pentagonal; arms 5 (to 50), not sharply distinct from central disc.....STARFISHES Cl. *ASTEROIDEA*
53(52) Body of distinct small central disc and 5 slender jointed arms..... .....BRITTLE STARS Cl. *OPHIUROIDEA*
54(51) No arms; body hemispherical, egg-shaped, or disc-like; many movable spines .....SEA URCHINS, SAND DOLLARS, ETC. Cl. *ECHINOIDEA*
55(48) Body elongate to worm-like, with soft leathery wall; no arms or spines; often with branched tentacles.....SEA CUCUMBERS Cl. *HOLOTHURIOIDEA*
56(67) Body soft, unsegmented, enclosed in limy shell or with ventral muscular foot, or with both; some with fleshy arms or tentacles
57(58) Shell with 2 valves, dorsal and ventral; a fleshy posterior peduncle for attachment.....LAMP SHELLS Ph. **BRACHIOPODA**
58(57) Shell of 1, 2 (lateral), or 8 parts, or none; a ventral muscular foot (or fleshy arms or tentacles); head usually present.....MOLLUSKS Ph. **MOLLUSCA**

AMPHINEURA SCAPHOPODA PELECYPODA GASTROPODA CEPHALOPODA

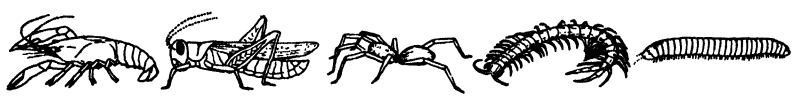
FIG. 17.—Phylum MOLLUSCA. The 5 Classes.

59(66). No arms with suckers; eyes small or none
60(61) Shell of 8 butterfly-like plates (rarely covered), surrounded by fleshy girdle; no tentacles.....CHITONS Cl. *AMPHINEURA*
61(60) Shell of 1 or 2 parts
62(63) Shell narrowly conical, open at both ends; foot conical.....TOOTH SHELLS Cl. *SCAPHOPODA*
63(62) Shell not narrowly conical
64(65) Shell of 1 valve, usually spirally coiled (some flatly conical, some lack external shell); head distinct; tentacles, 1 or 2 pairs; eyes, 1 pair.....SNAILS AND SLUGS Cl. *GASTROPODA*
65(64) Shell of 2 lateral valves, right and left, usually symmetrical; no head.....BIVALVES Cl. *PELECYPODA*
66(59) Head conspicuous, with 2 large eyes, and either 8 or 10 arms with suckers or many tentacles.....SQUIDS, OCTOPUSES, ETC. Cl. *CEPHALOPODA*
67(56) Body segmented (metameric), or with jointed appendages, or both
68(73) Form slender, worm-like: cuticle thin; no exoskeleton; some live in secreted tubes; appendages are setae (or none)...SEGMENTED WORMS Ph. **ANNELIDA**

OLIGOCHAETA POLYCHAETA HIRUDINEA

FIG. 18.—Phylum ANNELIDA. The 3 commoner Classes.

69(72) Setae present; somites distinct; no suckers
70(71) Setae many per somite, usually on fleshy lateral appendages (parapodia).....MARINE WORMS Cl. *POLYCHAETA*
71(70) Setae few per somite and in body wall.....EARTHWORMS, ETC. Cl. *OLIGOCHAETA*
72(69) Setae lacking; somites indistinct but many annuli; suckers at both ends or one at posterior end.....LEECHES Cl. *HIRUDINEA*
73(68) Body in firm segmented exoskeleton of chitin and with 1 (or 2) pair of jointed appendages per somite.....JOINT-FOOTED ANIMALS Ph. **ARTHROPODA**



CRUSTACEA INSECTA ARACHNOIDEA CHILOPODA DIPLOPODA

FIG. 19.—Phylum ARTHROPODA. Representatives of the 5 principal Classes.

74(81) Paired antennae present
75(76) Two pairs of antennae; paired appendages usually numerous, some often 2-branched (biramous); often with gills ...CRUSTACEANS Cl. *CRUSTACEA*
76(75) One pair of antennae; head, thorax, and abdomen more or less differentiated
77(78) Not more than 3 pairs of legs (on thorax) and commonly with 2 pairs (1 or none) of wings.....INSECTS Cl. *INSECTA*
78(77) With 15 or more pairs of jointed legs; body long
79(80) Appendages 1 pair per body somite.....CENTIPEDES Cl. *CHILOPODA*
80(79) Appendages mostly 2 pairs per somite.....MILLIPEDES Cl. *DIPLOPODA*
81(74) No antennae; body of cephalothorax and abdomen; segmentation often obscured; 4 pairs of walking legs.....SPIDERS, SCORPIONS, TICKS, ETC. Cl. *ARACHNOIDEA*

82(26) With lateral gill slits in pharynx or else body with internal spinal column of vertebrae, or both; nerve cord dorsal, single; heart ventral, if present; higher forms typically with 2 pairs of lateral fins or limbs.....
.....CHORDATES Ph. **CHORDATA**

83(88) No cranium, vertebrae, or brain

84(87) Body not compressed; no lateral <-shaped muscles

85(86) Body usually worm-like, with fleshy proboscis and collar.....
.....TONGUE WORMS Subphylum HEMICHORDATA

86(85) Body sac- or barrel-like or irregular; enclosed in a test.....
.....TUNICATES Subphylum TUNICATA

87(84) Body compressed; many <-shaped muscle segments.....
.....LANCELETS Subphylum CEPHALOCHORDATA

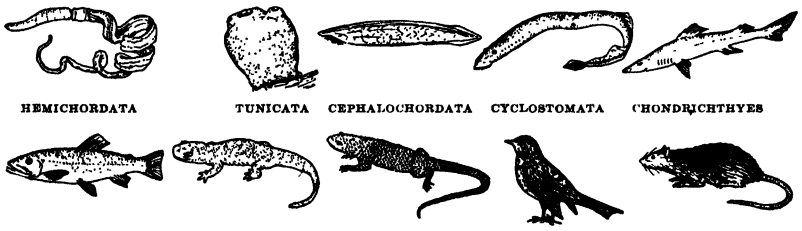


FIG. 20.—Phylum CHORDATA. Representatives of the 3 lower Subphyla, and of the 7 Classes of living vertebrates.

88(83) Cranium, vertebrae, and brain developed.....VERTEBRATES

89(90) Mouth circular; no true jaws or paired appendages.....
LAMPREYS AND HAG FISHES Subphylum AGNATHA Cl. *CYCLOSTOMATA*

90(89) Head distinct; mouth with jaws; body typically with 2 pairs of lateral appendages.....JAWED VERTEBRATES Subphylum GNATHOSTOMA

91(94) Skin with scales; paired fins usually present; gills beside pharynx

92(93) Scales minute, placoid, exposed; skeleton cartilaginous; gills 5 to 7 pairs in separate clefts; males with claspers.....
.....SHARKS, RAYS, ETC. Cl. *CHONDRICHTHYES*

93(92) Scales cycloid or ctenoid, often large, covered with epithelium; skeleton more or less bony; gills 4 pairs under common operculum.....
.....BONY FISHES Cl. *OSTEICHYTHYES*

94(91) Skin cornified; 2 pairs (or less) of limbs; nostrils connect to mouth cavity; lungs present (except in few amphibians).....LAND VERTEBRATES

95(96) Toes lack claws; skin soft, moist, and slimy, with no external scales.....
.....AMPHIBIANS Cl. *AMPHIBIA*

96(95) Toes with claws, nails, or hoofs; skin dry

97(98) Body covered with scales or scutes.....REPTILES Cl. *REPTILIA*

98(97) Body covered with feathers or hair

99(100) Body more or less covered with feathers; fore limbs are wings; mouth a toothless horny beak.....BIRDS Cl. *AVES*

100(99) Body usually more or less covered with hairs; females with paired ventral mammary glands.....MAMMALS Cl. *MAMMALIA*

PART II. THE ANIMAL KINGDOM

EXERCISE 18. **AMOEBA**

Phylum **PROTOZOA** Class *SARCODINA*

(Storer, "General Zoology," pp. 257–267)

The protozoans are mostly one-celled animals of microscopic size. Many kinds live in the sea, others in fresh waters, and some in the soil; still others are parasites that live on or in the bodies of other animals.

Free-living fresh-water amoebas occur on the bottom ooze of ponds or slow streams, on the slimy coating of leaves and stems in the water, and in the surface scum. If such materials are brought to the laboratory in water, kept a few days and searched, amoebas often will be found, along with various other microscopic organisms. Amoebas also can be reared in laboratory "cultures" started from such sources. Of the Genus *Amoeba* there are several species; *Amoeba proteus* (text, Fig. 12·2) is commonly studied because it is relatively large and active.

1. General structure. *Observe a demonstration of amoeba on a microscope. Then present a clean slide to the instructor and receive a drop of culture containing one or more living specimens. Add a few fibers of lens paper or bits of No. 1 coverglass for support, and gently lay on a clean coverglass. Using the middle power of the microscope and much reduced illumination, search for an amoeba by moving the slide slowly back and forth.* The animal is transparent, nearly colorless (faintly blue or gray), granular, irregular in shape, and almost motionless. *If necessary, ask the instructor for aid in searching or to verify your identification of the animal. Then study under high power. Later add culture fluid as needed to prevent drying of the preparation. Distinguish:*

Ectoplasm (thin clear layer surrounding cell body)
Endoplasm (darker, granular, inside ectoplasm)
Pseudopodia (blunt projections of cell body)
Food vacuoles (within endoplasm; of various sizes; contents surrounded by clear fluid)
Contractile vacuole (spherical; clear; forms and then disappears at intervals)
Nucleus (disc-like; often difficult to see)

Draw Fig. 1

2. Locomotion. *Watch the amoeba closely for several minutes.* Does it retain a constant shape? *Note the flowing movements of the ectoplasm and endoplasm, including the granules in the latter.* How does it change form? What is the rate of change? Which portion seems more fluid? Where is the ectoplasm thickest and thinnest? Watch for the formation of a blunt projection or pseudopodium (Gr. *pseudos*, false + *podos*, foot).

Does the animal move constantly in one direction? Does it always travel with the same part of its cell body forward? What happens if the amoeba meets an obstruction? This type of locomotion is termed "amoeboid movement." What cells of multicellular animals perform such movements? What happens if two or more pseudopodia are formed at the same time?

Draw Figs. 2 and 3

3. Feeding. *Watch for the taking in* (ingestion) *of food or disposal* (egestion) *of solid waste.* Do these occur at particular places on the cell body? Each bit of food while undergoing digestion is within a fluid-filled food vacuole. The progress of digestion is indicated by loss of distinct outline in the food and by decrease in size of the food particle and vacuole. What is the food of amoeba? How are food vacuoles moved about?

4. Contractile vacuole. *Locate the clear, fluid-filled spherical contractile vacuole that periodically fills, enlarges, and then is discharged outside the cell body. Time its frequency of emptying.* Does it occupy a constant position within the animal? What purpose does it serve? How are the functions of circulation, respiration, and excretion performed in an amoeba?

5. Irritability and response. *While watching through the microscope, tap the coverglass lightly with a needle and determine the reaction of the amoeba. Endeavor to see the amoeba while floating, creeping, and contracted.* How do the pseudopodia differ in these three conditions? If time permits, the instructor may give directions for experiments with simple chemicals on the amoeba.

6. Nucleus. *Search the endoplasm for the nucleus which is oval or disc-shaped and finely granular.* It is about the size of the contractile vacuole when the latter is largest. Special stained slides may be demonstrated to show the nucleus clearly.

7. Reproduction. *Watch for an amoeba dividing by binary fission, or examine stained preparations of this process, if available.*

8. Parasitic amoebas. *If available, examine a demonstration slide containing cysts of a parasitic amoeba.*

Is the amoeba a cell, an individual organism, or both? Why? What are the various metabolic processes occuring in an amoeba? What is meant by solation and gelation? What are some other species or groups of the Class SARCODINA? Are any of them important in human welfare? How is amoebic dysentery spread, and how may the disease be avoided? What protozoans have contributed to the formation of rocks and sediments in the earth's crust? Of what economic value are the FORAMINIFERA?

DRAWINGS

Fig. 1. Amoeba (about 70 mm. long). Draw and label the parts actually observed. Leave the ectoplasm clear and stipple the endoplasm lightly.

Fig. 2. Series of 5 to 10 numbered outline sketches (25 mm. long) at regular intervals (30 seconds to 2 minutes) to show the successive changes in form of an amoeba; indicate movements of the animal and its endoplasm by arrows.

Fig. 3. Tip of a pseudopodium under high magnification (50 mm. long); distinguish, if possible, the plasmalemma and liquid ectoplasm, and the plasmagel and plasmasol of the endoplasm.

EXERCISE 19. EUGLENA

Phylum **PROTOZOA** Class *MASTIGOPHORA*

(Storer, "General Zoology," pp. 267–273)

The various kinds of flagellates (MASTIGOPHORA) have 1, 2, or more whip-like flagella that serve for locomotion. Many inhabit fresh, foul, or salt waters; others live in soil; and a variety occur in plants and animals, some as commensals or symbionts and others as disease-producing parasites. Species of green flagellates of the Genus *Euglena* inhabit stagnant pools, ditches, and streams, sometimes being so abundant as to produce a green surface bloom or green spots on the bottom ooze. They may be collected readily in such places and also may be reared in special culture solutions. *Euglena viridis*, *E. gracilis*, and *E. deses* are commonly used for study.

1. General structure. ***A.*** *Observe a demonstration of euglena. Then present a clean slide and obtain a drop of water containing euglenas; lay on a cover glass and study under medium and high magnification.* The euglena is spindle-shaped and green. *Distinguish:*

Anterior end (blunt)
Posterior end (tapered)
Flagellum (anterior, thread-like; use reduced oblique light or stain to distinguish)
Cell gullet (clear, at anterior end)
Reservoir (clear, behind gullet)
Contractile vacuoles (small; number?)
Stigma (small, red)
Nucleus (central)
Pellicle (thin covering of cell)
Ectoplasm (thin, under pellicle)
Endoplasm (within ectoplasm)
Chloroplasts or chromatophores (green, contain chlorophyll)

Darkfield illumination (p. 14) will aid in seeing some of these features.

B. *If available, examine stained preparations to identify the paramylum bodies* (starch-like) *and pyrenoid bodies.*

Draw Fig. 1

2. Locomotion. *Distinguish three kinds of movement:* (*a*) swimming, with swaying motion; (*b*) spiral crawling; and (*c*) worm-like contractions and expansions of the cell body known as "euglenoid movement." How does euglenoid movement differ from amoeboid movement? Is one end of the euglena regularly directed forward? Is the cell body of essentially constant shape? Of what service may the pellicle be in respect to shape? How does the flagellum serve in locomotion?

Draw Fig. 2

3. Physiology. What is the food of euglena and how is it obtained (text, p. 268)? Is such nutrition holozoic, holophytic, or saprozoic? Of what service is the chlorophyll? What is photosynthesis? What are the paramylum bodies and how are they related to metabolism? What is the presumed function of the stigma? Where do euglenas concentrate in a culture? What is their response to light? In what respects is the euglena animal-like? In what respects is it plant-like?

4. Reproduction. *A. Watch for instances of longitudinal binary fission in your material, or examine a stained demonstration if available.*

B. Examine material from an old standing culture for encysted euglenas or consult a demonstration slide; distinguish the cyst wall. What other structures seen in an active euglena are present? How many individuals are present within a cyst? Of what advantage is cyst formation to such an animal?

Draw Figs. 3 and 4

5. Symbiotic or parasitic flagellates. *Examine demonstration slides of other flagellates if available.*

What is the role of some of the marine flagellates that abound in the sea? Of what service are the flagellates in certain termites to their hosts? What are some important flagellates of animals and man? What are two or three that produce serious human diseases?

DRAWINGS

Fig. 1. Euglena (100 mm. long). Show and label all parts seen.
Fig. 2. Three or more outline sketches to show euglenoid movement.
Fig. 3. Binary fission in euglena.
Fig. 4. Encysted euglena. Label cyst wall and any other parts seen.

EXERCISE 20. VOLVOX

Phylum **PROTOZOA** Class *MASTIGOPHORA*

(Storer, "General Zoology," pp. 269–270)

In most species of protozoans each individual is separate and distinct, but in some kinds of flagellates and ciliates a few or many individuals remain attached to one another after cell division and thus form colonies. In different types the colonies are chain-like (linear), tree-like (branched), or spherical. *Pandorina*, *Eudorina*, *Volvox*, and some other green flagellates of fresh waters grow as small spheres with the individuals embedded in a gelatinous exterior wall (text, Fig. 12·14). *Volvox* is of especial interest in being composed of two kinds of cells that differ in structure and function. The several thousand "somatic" or body cells of the exterior wall synthesize food and serve in locomotion but all finally die, whereas the "germ" cells serve only for continuing the species by sexual reproduction. *Volvox* thus suggests a possible path in evolution from PROTOZOA to METAZOA and in the evolution of separate sexes.

1. The colony. *Examine a volvox under low magnification, either living in a drop of water on a slide or on a prepared slide. Use special care not to crush the coverglass. Identify:*

Gelatinous matrix (colony wall)
Central cavity
Somatic individuals (each with nucleus, chromataphore or chloroplast, stigma, 2 flagella)
Protoplasmic threads (connect individual cells)

Draw Figs. 1 and 2

2. Asexual reproduction. *Find scattered cells that lose the flagella and enlarge as spherical parthenogonidia.* These multiply within the hollow center of the mother colony to produce spherical masses of small cells termed "daughter colonies"; later they escape to begin new colonies.

Draw Fig. 3

3. Sexual reproduction. After several asexual generations some non-flagellated cells enlarge and become macrogametes ("ova"), and others in the same or a different colony (depending on the species of *Volvox*) divide repeatedly to become flat bundles of up to 128 slender microgametes ("sperm"). Both types are discharged into the hollow center of the colony, an "ovum" is penetrated and fertilized by a "sperm," and the resulting zygote secretes a brownish-red smooth or spiny cyst about itself. After the colony dies, the zygote survives the winter and starts a new colony in the spring.

Draw Fig. 4

What are the arguments for classifying volvox as an animal (protozoan) or a plant (alga)? What are the relative advantages and dis-

advantages of colony life? What is the effect on the component cells? What is physiological division of labor? What is death in respect to the individual, the colony, and the species?

DRAWINGS

Fig. 1. Entire volvox (60 mm. in diameter).

Fig. 2. Few somatic cells (each 25 mm. in diameter); show and label parts identified.

Fig. 3. Parthenogonidia. Show as attached to part of colony wall.

Fig. 4. Macrogamete, microgamete, and zygote within cyst.

EXERCISE 21. SPOROZOANS

Phylum **PROTOZOA** Class *SPOROZOA*

(Storer, "General Zoology," pp. 273–277)

The SPOROZOA lack structures for locomotion; some move by changes in shape of the cell body. All are parasites of other animals, and absorb food from their hosts. They reproduce by multiple asexual fission (schizogony) and also sexually by gametes. Some produce oöcysts that are spread from one host to another. Sporozoans are found by searching the organs and tissues of host animals, and they may be studied either in fresh or stained preparations.

1. Gregarina. *From a live larva of the mealworm beetle (Tenebrio molitor), cut off the head and last somite, pull out the digestive tract, remove any adhering parts of the fat body, and examine the gut on a slide under low magnification. Any gregarines present will appear as dark bodies longer than wide. If found, use a sharp scalpel to slit and spread open the gut, flood with* 0.9 *per cent salt solution, and lay on a coverglass; examine under middle power.* Individual gregarines may unite in chains (syzygies). *Distinguish:*

Cell membrane (firm)
Nucleus
Ectoplasm (dense)
Endoplasm (more fluid and composed of anterior protomerite and posterior deutomerite)

Draw Fig. 1

2. Monocystis. *From a fresh earthworm, tease out parts of the seminal vesicle in* 0.7 *per cent salt solution on a slide and add a coverglass. Search for spherical cysts containing* 2 *cells or many spindle-shaped cells and also for the active spindle-shaped stages; the latter may be free or else within masses of earthworm tissue from which tails of spermatozoa protrude.*

Draw Fig. 2

3. Other sporozoans. *Examine demonstrations of any species provided, such as Eimeria, which produces the disease coccidiosis, or Plasmodium, responsible for malaria in man, mammals, and birds.*

Draw Figs. 3 and 4

As compared with amoeba and euglena, is the structure of a sporozoan simpler? Why? What are some advantages and disadvantages of the parasitic manner of life in respect to the parasite?

What are some disease-producing sporozoans? Are they of economic importance and why? What is the life cycle of *Eimeria stiedae* in the rabbit? What are the principal stages in the life cycle of the malarial parasite? How do *Eimeria* and *Plasmodium* differ in mode of transmission from host to host? What means may be used to prevent malarial infection? What treatment is possible for malarial patients? What general principle is the basis for treatment of a host for parasitic infection?

DRAWINGS

Fig. 1. *Gregarina* (25 mm. long). Label.
Fig. 2. *Monocystis*. Show all stages found.
Fig. 3. *Eimeria*. Oöcysts or other stages.
Fig. 4. *Plasmodium* in red blood cell (make cell 25 mm. in diameter).

EXERCISE 22. PARAMECIUM

Phylum **PROTOZOA** Class *CILIATA*

(Storer, "General Zoology," pp. 277–288)

Ciliates are distinguished by the presence of fine movable projections of the cell body known as ***cilia*** which serve for locomotion and food capture. (Some cells in multicellular animals also are ciliated.) Some types of ciliates are covered uniformly with cilia, others have them restricted to parts of the cell body, and some have groups of cilia clumped as cirri. Ciliates also have other "cell organs" or organelles that perform special physiological functions (text, Fig. 12·29).

Certain ciliates abound in fresh, foul, and salt waters, others inhabit the soil, and various types live on or in other animals as either commensals or parasites (text, pp. 286–288; 291). The ciliates are also called "infusoria" because many kinds grow abundantly in an infusion made by boiling a little grass or hay in water; such a culture fluid promotes the growth of bacteria on which many ciliates feed. Some species of ciliates are common in sewage-polluted waters which swarm with bacteria.

Ciliates of the Genus *Paramecium*, particularly *Paramecium caudatum* (called the "slipper animalcule" because of its shape), serve for a beginning study. They may be cultured easily in the laboratory. In a culture the paramecia are commonest at the top (where bacteria and oxygen are most abundant), appearing as slender moving

specks visible to the unaided eye. Ciliates swim rapidly and for careful study in a drop of culture water must be slowed down. Darkfield illumination aids in showing many of their structural features, especially the trichocysts, vacuoles, and cilia.

1. General structure. *Place a drop of culture containing paramecia on a slide and add material to slow down the animals, as directed by the instructor; lay on a coverglass, and observe a quiet but undistorted individual. Identify:*

Anterior end (blunt)
Posterior end (tapered)
Pellicle (thin, covers cell body)
Cilia (over surface of cell body)
Trichocysts (slender, under pellicle)
Ectoplasm (thin, clear; beneath pellicle)
Endoplasm (granular, fills cell body within ectoplasm)
Food vacuoles (in endoplasm; clear; contain particles of food)
Oral groove (diagonal, from anterior end)
Cell mouth (at posterior end of oral groove)
Cell gullet (from cell mouth)
Contractile vacuoles (number? location?)
Macronucleus } see
Micronucleus } Par. 6

Draw Fig. 1

Is the animal symmetrical? How do the anterior and posterior ends differ? What is the direction of the oral groove? Which is the ventral or oral surface of the animal? Are cilia present uniformly over the entire cell body? How are they arranged? Where are they longest? How do they move? How do they accomplish locomotion?

2. Feeding. *Spread a drop of dilute water solution of neutral red on a clean slide, allow it to dry completely, add a drop of paramecium culture and lay on a coverglass, and immediately observe a quiet individual under medium and high magnification.* (Powdered carmine suspended in water or yeast also will serve in the study of ingestion.) How do the cilia in the oral groove act to take in food? Where and how are food vacuoles formed? What is the course of the colored food vacuoles within the endoplasm? How rapid is the feeding process? How often are vacuoles formed? How is the rate of feeding related to the nature of the food? What changes occur within the food vacuoles during digestion? The "cell anus" can be located only when indigestible material is egested.

3. Contractile vacuoles. *A. Study a slightly dried preparation in which the coverglass presses lightly on the paramecia.* What is the number and location of the contractile vacuoles? Is their location constant? *Time the successive pulsations in one vacuole. Study the relation of the radiating canals leading to a forming vacuole. If possible, warm and then cool the slide and observe the effect on the rate of pulsation.*

B. To a fresh drop of culture under a coverglass, add a dense suspension made from dry India ink (carbon rubbed up in water) *and watch the discharge of a vacuole.*

4. Pellicle. ***A.*** *With a fresh preparation, draw a drop of* 35 *per cent alcohol under the coverglass;* the pellicle separates as a blister over the ectoplasm.

B. *Combine a drop of paramecium culture and a drop of nigrosin solution on a slide; spread gently and allow to dry completely.* Under high magnification the surface depressions in the pellicle are seen to be filled with the black dried nigrosin. What is the surface pattern? How is this related to the cilia?

5. Trichocysts. *In a quiet normal paramecium under high magnification observe the slender oval trichocysts just under the pellicle. Draw a drop of dilute methyl green solution or Delafield's hematoxylin stain under the cover slip and watch for the discharge of trichocysts to form a mass of fine threads about the animal.* What purpose may they serve?

6. Nuclei. The large macronucleus may sometimes be seen in living paramecia; it readily stains bluish green with methyl green (see Par. 5). Demonstration slides, prepared with acetocarmine or other stains, will show the micronucleus (1 in *P. caudatum*, 2 in *P. aurelia*). What are the respective functions of the two types of nuclear structures?

7. Behavior. ***A.*** *Place a large drop of paramecium culture on a slide* (omit the coverglass) *and examine under low magnification. Watch the course of an individual.* What sort of a route does it travel? *Distinguish the swinging and rotating motions.* How does it accomplish progress in a straight line? What happens when it encounters a solid object? *Analyze the stages in the avoiding reaction.*

B. The instructor may arrange for experiments on reactions of paramecium to (1) salts, (2) acids, or (3) temperature differences.

8. Reproduction. ***A.*** Individuals dividing by binary fission may be found at times in cultures. *If not seen, examine demonstration slides.* What happens to the macronucleus? To the micronucleus? What is the plane of fission?

Draw Fig. 2

B. *Examine stained preparations showing conjugation* (see text, Fig. 12·26). What changes take place in the macronucleus and in the micronucleus during conjugation?

Draw Fig. 3

DRAWINGS

Fig. 1. Entire paramecium (100 mm. long); show and label parts mentioned in Par. 1, and the nuclei (Par. 6).

Fig. 2. Paramecium undergoing fission (50 mm. long); show nuclei and contractile vacuoles.

Fig. 3. Two conjugating paramecia (50 mm. long).

EXERCISE 23. MICROSCOPIC ANIMALS OF FRESH WATER

Water from any pool with aquatic vegetation will usually reveal, under medium or high magnification, many microscopic organisms—plants, and various animals belonging to several phyla and classes of the Animal Kingdom. Collectively these constitute a "microcosm," or small world. The algae, diatoms, and chlorophyll-bearing flagellates produce, by photosynthesis, from the water, carbon dioxide, and dissolved minerals, the organic substances that constitute their one-celled bodies. They in turn serve as the beginning stages in various food chains (text, p. 172), which eventually lead up to large fishes, birds, and mammals and so in part to the nourishment of mankind. Reservoirs of water for human consumption are examined regularly by trained microscopists, and when certain organisms are found that impart unpleasant tastes the water must be treated to remove them. Water from reservoirs and rivers that is intended for domestic use is filtered through sand beds and chlorinated; these processes kill and remove such animals as are mentioned below, and also many bacteria. For details about certain animals consult the text and classifications in Chaps. 11, 12, 14, 16 to 18, 22 to 24. See also the Key in this manual and the reference works by Ward and Whipple, Pratt, and others listed in text, Chap. 1.

1. Fresh-water culture. *Put some water from a pond or stream into a clean glass container* (jar, tumbler, or finger bowl). *Add a small amount of grass, hay, wheat, manure, or meat* (instructor will demonstrate), *which will decay and provide material for start of a food cycle* (text, Fig. 7·4). *Cover loosely to reduce evaporation. Examine regularly once a week; with a clean pipette* (one never used in formalin) *withdraw samples in turn from the bottom, surface, and center; then study each under the compound microscope.*

Begin Fig. 1

Some organisms may be restricted to certain parts of the culture because of their particular needs for food or oxygen, or for other reasons. As the culture ages there will often be a succession of animals, analogous to the sequence in blossoming of different garden flowers throughout the season.

Some groups and animal types common in fresh-water samples or cultures include:

Phylum **PROTOZOA.** PROTOZOANS. Structure unicellular, no true organs.

Class *SARCODINA.* With pseudopodia. *Amoeba; Arcella,* with brown hemispherical shell; *Difflugia,* shell of cemented sand grains; *Actinophrys,* pseudopodia many, slender, stiff, and radiating.

Class *MASTIGOPHORA.* FLAGELLATES. With flagella; some green, like *Euglena* and *Volvox,* others colorless; travel by swaying movements (text, Figs. 12·14, 12·15).

Class *CILIATA.* CILIATES. With cilia (text, Fig. 12·28). *Paramecium; Didinium; Stentor,* trumpet-shaped, contractile; *Spirostomum,* to 4.5 mm. long and very slender; *Colpoda,* kidney-shaped, flattened; *Bursaria; Lacrymaria,* like a long-necked flask; *Vorticella,* vase-like, on long contractile stalk.

Phylum **COELENTERATA.** Hydras may occur on leaves in water (text, Fig. 14·12).

Phylum **PLATYHELMINTHES.** FLATWORMS. Slender, flat, thin; usually with a head region; glide smoothly. *Euplanaria*, etc. (text, Fig. 16·1).

Phylum **NEMATHELMINTHES.** ROUNDWORMS. Body round in section, slender and tapering toward ends, unsegmented; no cilia; movements whip-like, abrupt, curving and straightening only.

Phylum **TROCHELMINTHES.** Class *ROTIFERA*. ROTIFERS (text, Fig. 18·4). Transparent; anterior end with disc (or 2) of cilia moving so as to appear wheel-like; often a tail-like movable "foot."

Phylum **ANNELIDA.** SEGMENTED WORMS. Body of obvious segments or somites much alike, with bristles (setae) on each somite.

Phylum **ARTHROPODA.** Body variously of somites, often unlike in different parts, and with jointed appendages.

Class *CRUSTACEA*. CRUSTACEANS. Two pairs of antennae; commonly with dorsal carapace of 1 or 2 parts. *Daphnia*, water flea, to 2 mm. long and with a mid-dorsal eye.

Class *INSECTA*. INSECTS. One pair of antennae. Some small larvae.

Class *ARACHNOIDEA*. No antennae. Includes water mites with 4 pairs of legs.

Which types are most abundant in early cultures? Which types later? What kinds of locomotion are most effective? Why are rotifers and nematodes often present in old protozoan cultures? How do these various microscopic animals become distributed?

DRAWINGS

Fig. 1. Microscopic animals, 10 or more, including 5 protozoans (make each sketch 25 mm. or more in length); identify by name and give date seen and approximate size (use *Paramecium*, 0.2 mm. long, as a standard).

EXERCISE 24. SPONGE

Phylum **PORIFERA** Class *CALCAREA*

(Storer, "General Zoology," pp. 293–300)

Sponges are the lowest of multicellular animals; the "body" has many pores and consists of a simple loose tissue structure supported by a framework of minute or microscopic rods or spicules. Sponges are aquatic and live attached to submerged rocks, weeds, wood, and animal shells; all live in salt water, except for a few gelatinous-bodied species inhabiting fresh waters. Shallow-water species are collected easily by hand and deep-water forms by dredges. The sponge commonly studied is known as "grantia" (Genus *Sycon*).

1. External features. *Examine an entire preserved specimen in water and identify:*

Body
Base (attached end)
Osculum (opening at top)
Spicules (in external wall; bristly)
Ostia or pores (in body wall)
Bud (if present)

Draw Fig. 1

2. Internal structure. *Study a preserved specimen split lengthwise and placed in water* (or a thin hand-cut, dried section); *use a hand lens or low-powered microscope. Identify:*

Spongocoel (central cavity or "cloaca")
Osculum (exit from spongocoel)
Ostia (pores in body wall)
Spicules
Incurrent canals (connect to ostia)
Radial canals (flagellated, connect to spongocoel)

Draw Fig. 2

3. Skeleton. *In a dried section notice the arrangement of spicules. Then examine isolated spicules obtained by boiling a sponge in sodium hydroxide* (NaOH) *solution. Find two kinds each of monaxon and triaxon spicules.*

Draw Fig. 3

4. Cellular structure. *Study a stained cross section under medium and high magnification; identify:*

Epidermis (external, thin)
Ostia (open into incurrent canals)
Incurrent canals
Radial canals (open into spongocoel)
Choanocytes or flagellated cells (in radial canals; each cell with collar and flagellum)
Gastral epithelium (lining spongocoel)
Mesenchyme (gelatinous)
Amoebocytes (in body wall)
Spicules (if present)

Draw Fig. 4

How do the spicules form a skeleton? What other purpose may they serve? What are the paths of water currents in grantia? What produces these currents? What essential functions do they aid?

How does a sponge obtain food? Where and how is food digested? Is the spongocoel a digestive cavity? Why? What advance does a sponge show over a colonial protozoan?

What are some relations between living sponges and other animals? What is a "bath" sponge? How are sponges cultured?

DRAWINGS

Fig. 1. Entire grantia (50 mm. long), external view.

Fig. 2. Grantia in median section (70 mm. long). Show details of the canals only in a small portion.

Fig. 3. Spicules, monaxon and triaxon.

Fig. 4. Part of body wall of sponge in microscopic section, and enlarged sketch of one choanocyte.

EXERCISE 25. HYDRA

Phylum **COELENTERATA** Class *HYDROZOA*

(Storer, "General Zoology," pp. 301–310)

The coelenterates are the lowest multicellular animals with distinct layers of cells organized as tissues and with a definite digestive cavity. The individual coelenterate is either an attached cylindrical polyp, single or in colonies, or else a free-floating bell-like medusa (text, Fig. 14·11); both types appear in the life cycle of many species. All coelenterates are aquatic, and all but a few are marine.

The hydras are slender solitary polyps, 10 to 30 mm. long, that live in cool fresh waters but often are local in occurrence. There are several types, white, brown, and green; the last is colored by green algae (zoochlorellae) in its inner cells. Hydras may be collected by bringing soft leafy aquatic plants in water to the laboratory; the animals will be found on the leaves and other surfaces. They also may be grown in aquaria containing clear cool water and supplied with minute crustaceans for food.

1. General structure. *Study a living hydra in a watch glass under low magnification. Use enough water* (5 mm. depth or more) *to enable the hydra to move freely; do not contaminate the water with dirty instruments. Identify:*

Body column
Enteron (cavity of body and tentacles; use transmitted light to see)
Basal disc (for attachment)
Tentacles (number?)
Hypostome (at top of column)
Mouth (in hypostome)
Bud (if present)
Nematocysts (stinging cells; minute, in groups on tentacles)
Cell layers (2; in body and tentacles)

Draw Fig. 1

What type of symmetry is present?

2. Behavior. ***A.*** *Observe the normal movements of the column and tentacles. Try to see an individual in locomotion.*

B. *Tap the dish lightly and observe the reaction of the hydra. Twirl the dish slowly.* How does the animal respond? *Wash a dissecting needle carefully, then use it to touch the body and tentacles very gently at different points; allow the hydra a brief rest between tests.* What parts are most sensitive? Is the response proportional to the stimulus? Are contractions of the body and tentacles independent or coordinated? If coordination is shown, what structural features could exercise control (text, p. 306)?

Draw Fig. 2

3. Feeding. *Use a large hydra kept without food for a day or more. Bring some water fleas (Daphnia) or small bits of animal flesh close to a tentacle while the hydra is attached. Watch throughout the procedure under low magnification.* How is food captured? What are the steps in ingesting the food?

4. Nematocysts. *Mount a living hydra on a slide, add thin strips of clean glass or wood to support a coverglass, and lay on the latter. Under the compound microscope find, on the tentacles, groups of cnidoblast cells, each containing a stinging nematocyst* (text, Fig. 14·4). *To discharge the nematocysts* (*a*) *tap the coverglass, or* (*b*) *introduce a drop of safranin solution under the coverglass. Search for different types of nematocysts.*

Draw Fig. 3

5. Cellular structure. *Study stained cross or longitudinal sections of hydra under medium and high magnification. Identify the two body* (germ) *layers and types of cells in each, also the thin noncellular mesoglea between the layers:*

Epidermis (external, with epithelio-muscular cells, interstitial cells, and nerve cells)
Gastrodermis (internal, with epithelio-muscular cells, gland cells, flagellated cells, and interstitial cells)
Food vacuoles (in some gastrodermal cells)
Muscle fibrils (in epithelio-muscular cells)

Draw Fig. 4

How does the enteron of hydra differ from the central cavity of a sponge? From the digestive tract of a frog? How do the contractile fibrils in the epithelio-muscular cells act to lengthen the body? To shorten the body? What is the function of the mesoglea? How are respiration and excretion performed?

6. Regeneration. *In a clean watch glass with water place one or more hydras. When extended, use a carefully washed scalpel to cut each in 2 or 3 pieces. Cover with another watch glass and examine at succeeding laboratory periods. Make dated outline sketches of the fragments as cut and in stages of regeneration.*

Begin Fig. 5

7. Reproduction. ***A.*** *Examine a living or preserved hydra showing a lateral bud* (asexual reproduction). From what tissues (cell layers) does the bud develop? How is it nourished? How does a bud become separated from the original hydra?

B. *Study living specimens or stained slides showing sex organs.* (1) The conical male gonads (spermaries) are usually distal on the column and contain many small sperm cells, later released by rupture of the gonads. (2) The female gonads (ovaries) usually are broader, rounded, and nearer the basal disc; each contains one egg. From what cells do the gonads develop? What is sex? Are the sexes separate (dioecious) or are both represented in one individual (monoecious) in the specimens examined?

Draw Fig. 6

DRAWINGS

Fig. 1. Entire hydra (column 70 mm. tall), exterior appearance, extended. Show a bud if present. Label parts named in Par. 1.

Fig. 2. Contracted hydra in outline (same scale as Fig. 1); show only the column and tentacles.

Fig. 3. Nematocysts (40 mm. long). Show and label 2 or more types.

Fig. 4. Cross section of column (70 mm. in diameter); label parts named in Par. 5.

Fig. 5. Series of outline sketches showing cut specimens and stages in regeneration. Date each figure.

Fig. 6. Sex organs, male and female.

EXERCISE 26. COLONIAL HYDROID

Phylum **COELENTERATA** Class *HYDROZOA*

(Storer, "General Zoology," pp. 311–312)

Many members of the Class HYDROZOA grow as colonies of minute polyps, others are medusae of some size (text, Figs. 14·1, 14·7), and most of the species include both polyp and medusa stages in their life cycles. If a hydra were to produce great numbers of buds that remained attached to one another, the result would resemble a hydroid colony. The colonies are mossy or feathery growths attached to rocks, piling, seaweeds, and shells in the sea. Many occur in shallow coastal waters and may be collected easily at low tide; others at greater depths are obtained by dredging. Specimens are preserved in formalin or alcohol, but to obtain expanded polyps they must be treated first with some narcotizing chemical such as chloretone. For detailed microscopic study, small fragments of colonies and the free medusae are fixed, stained, and mounted on slides.

1. General structure. *Examine entire colonies as preserved in museum jars to see the general growth form. Then study, at low magnification, part of a colony (Obelia* or other common type) *in water in a watch glass, or on a stained slide. Identify:*

Hydranths or feeding polyps (many)
Gonangia or reproductive polyps (fewer, long; contain medusa buds)
Perisarc (transparent noncellular covering)
Coenosarc (granular protoplasm connecting polyps)
Enteron (in polyps and coenosarc)
Hydrorhiza (root-like, attaches colony to substratum)

Draw Fig. 1

The column of each polyp connects to others by the canal (enteron) in the coenosarc (of epidermis and gastrodermis). The perisarc forms

a common support and covering which is expanded as a cup-shaped ***hydrotheca*** about each hydranth and a cylindrical cover or ***gonotheca*** over each gonangium.

2. Hydranth. *Study an expanded and stained feeding polyp under medium and high magnification. Note its likeness to a hydra. Identify:*

Body column
Hypostome
Mouth
Tentacles (solid, with nematocysts)
Enteron
Epidermis
Gastrodermis
Hydrotheca

Draw Fig. 2

3. Gonangium. *Find early bud stages of the reproductive polyp and also older stages containing medusa buds. Identify:*

Gonotheca (transparent, noncellular covering)
Blastostyle (hollow central stalk, continuous with coenosarc)
Medusa buds (knob- or saucer-like, on blastostyle)

Draw Fig. 3

4. Medusa. *In life the medusa floats freely and is cup- or bell-shaped, but may be distorted or turned inside out in prepared material. Identify:*

Bell
Tentacles (around margin, with nematocysts)
Manubrium (central stalk, under bell)
Mouth (at end of manubrium)
Enteron
Radial canals (out from enteron)
Ring canal (in margin of bell)
Gonads (on radial canals, if present)
Epidermis
Gastrodermis
Mesoglea (between cell layers on bell)

Draw Fig. 4

What is meant by division of labor between the two types of polyps? How is food distributed to medusa buds? How do the medusae escape from the gonangium?

What two types of reproduction are shown in a colonial hydroid? What is meant by alternation of generations or metagenesis? What is the life cycle of *Obelia?*

How does the organization of a colonial hydroid differ from that in a colonial protozoan?

DRAWINGS

Fig. 1. Small portion of hydroid colony, natural size; show hydrorhiza.

Fig. 2. One hydranth (50 mm. long) and part of supporting stalk; show and label parts named in Pars. 1 and 2.

Fig. 3. One gonangium (50 mm. long); label.

Fig. 4. Medusa (70 mm. in diameter); show and label parts named in Par. 4.

EXERCISE 27. JELLYFISH

Phylum **COELENTERATA** Class *HYDROZOA* or *SCYPHOZOA*

(Storer, "General Zoology," pp. 313–315, 319)

Jellyfishes are medusae from one inch to several feet in diameter and of bell or umbrella shape. The body substance is mostly of soft jelly-like mesoglea. The animals float or swim freely in the sea and capture small invertebrates as food by use of the nematocysts. Some species are common in coastal waters. Specimens are collected by use of nets and preserved in formalin for study. Live jellyfishes are difficult to keep, even in large seaside aquaria.

Gonionemus is a jellyfish about 20 mm. in diameter that belongs to the Class HYDROZOA. *Aurelia* is another, up to 300 mm. in diameter, belonging to the Class SCYPHOZOA. Both are common in coastal waters, and both have minute polyp stages in their life cycles.

1. General structure. ***A.*** *Examine a specimen in a dish of water; handle carefully to avoid damaging. Identify:*

Aboral surface or exumbrella (convex)
Oral surface or subumbrella (concave)
Manubrium (central stalk on oral surface)
Mouth (at end of manubrium)
Velum (shelf-like rim inside margin of bell; none in *Aurelia*)
Tentacles (many on margin of bell; with nematocysts)
Enteron ("stomach")
Radial canals (4)
Ring canal (in margin of bell)
Mesoglea
Gonads (4, in radial canals)
Pigment spots (at bases of tentacles)
Statocysts (sac-like organs of equilibrium between tentacles)

B. The following additional structures are present in *Aurelia* (which lacks the velum and manubrium):

Oral arms (4, about mouth)
Labial tentacles (on arms)
Gastric pouches (off enteron)
Additional canals (interradial, perradial)
Gastric filaments (in gastric pouches)
Lappets (8 pairs, in margin of bell)
Sense organs (between lappets; each with eyespot, statocyst, and sense pits)

Draw Figs. 1 and 2

How does a jellyfish swim? How is food captured? Where is it digested? How is it distributed through the body? Why does a medusa have more sensory organs than a polyp? What is the life cycle of *Aurelia*? When and where does asexual reproduction occur? How does the life cycle differ from that of a colonial hydroid?

DRAWINGS

Fig. 1. Entire jellyfish (100 mm. in diameter), oral view; if *Aurelia* is used, show canals only in one quarter.

Fig. 2. Sense organ and adjacent tentacles.

EXERCISE 28. SEA ANEMONE

Phylum **COELENTERATA** Class *ANTHOZOA*

(Storer, "General Zoology," pp. 315–318)

The sea anemones and the related corals are exclusively inhabitants of the sea. They are all polyps, but differ in several respects from hydra and other hydroids (Exercises 25, 26; text, Fig. 14·11). Most anemones live attached by the basal or pedal disc to rocks or other firm objects, but they can creep slowly. Some have much of the body buried (text, Fig. 14·1). The corals and some other anthozoans secrete external housings in which the individual polyps are sheltered. Some anemones can be kept in salt-water aquaria with considerable success, and various species thrive in public aquaria of coastal towns supplied with sea water. *Metridium dianthus* is a type common on North American coasts that serves for laboratory study.

1. External features. *Examine an entire preserved specimen in water and identify:*

Body column Pedal disc Oral disc Mouth Tentacles

Draw Fig. 1

2. Internal structure. *Immerse in water half of a specimen split lengthwise through the gullet, and find:*

Mouth (on oral disc)
Gullet (leads down from mouth)
Siphonoglyph (down side of gullet)
Enteron (interior cavity)
Septa or mesenteries (radial, dividing enteron)
Septal filaments (thick, convoluted, on edges of septa)
Acontia (slender ends of filaments)
Gonads (bead-like, on edges of septa)
Muscles (in body wall and on septa)
Ostia (openings in septa below oral disc)

Draw Fig. 2

3. Cross section. *Study cross sections (a) through the gullet and (b) through the gonads. These may be made by hand from large anemones or prepared microsections that are stained and mounted on a slide. Determine the relationships of the different types of septa* (primary, secondary, etc.). *Find cross sections of retractor muscles on septa. Distinguish epidermis, gastrodermis, and mesoglea.*

Draw Figs. 3 and 4

What is the function of each structure mentioned above? What changes in body shape are produced by the retractor muscles? How is the body elongated? Of what advantage is the tough epidermis? Of what special service are the septa? The siphonoglyphs? What is the food? How is it captured and ingested?

How does the sea anemone reproduce? Is it monoecious or dioecious? What is the life cycle? Is there a medusa stage?

In what ways are hydra, the colonial hydroid, the jellyfish, and the sea anemone alike? How do they differ from one another?

What is a coral? A coral reef? How are reefs formed? Where do they occur today?

DRAWINGS

Fig. 1. Entire sea anemone (50 mm. tall), in lateral view, slightly from oral side; show and label parts named in Par. 1.

Fig. 2. Median view of split anemone (70 mm. tall); include parts listed in Par. 2.

Fig. 3. Cross section through gullet (70 mm. in diameter).

Fig. 4. Cross section through gonads (70 mm. in diameter); if stained slide is available, show also some cellular detail of a gonad.

EXERCISE 29. PLANARIA

Phylum **PLATYHELMINTHES** Class *TURBELLARIA*

(Storer, "General Zoology," pp. 326–331)

Planarians (text, Figs. 16·1–5) are soft thin flat worms, 2 to 20 mm. long. Species of *Euplanaria* are commonly reddish brown, and those of *Procotyla* (*Dendrocoelum*) are whitish and translucent. They inhabit ponds and slow streams of pure cool fresh water and cling or crawl on submerged objects. Individuals may be gently gathered from stones, sticks, or leaves on the bottom and placed in a bottle of water. Pieces of meat or liver placed in such waters will often attract numbers of the worms which may then be washed into a bottle. In the laboratory, planarians should be kept in dishes with water from their native habitat that contains some green algae or duckweed to supply oxygen, and they should be protected from direct sunlight or heat. Specimens for microscopic study of the internal organs must be flattened under pressure, fixed, stained, and prepared either as whole mounts or sections.

1. Form. *Place a live planarian in some water in a watch glass and observe under a hand lens or low-power microscope.* Is the body segmented? What type of symmetry is present? Which end habitually moves forward? Which surface keeps in contact with the substratum or surface on which the animal rests or moves? *Distinguish, in relation to locomotion:*

Anterior end	Longitudinal axis	Dorsal surface
Posterior end		Ventral surface

Is the planarian to be compared with the hydra or with the frog in respect to these parts? What are the relative advantages of bilateral versus radial symmetry?

2. Locomotion. *A. Gently turn the animal over by use of a narrow slip of paper.* How does it right itself? How is locomotion accomplished?

Distinguish between gliding movements and muscular movements. Can waves of muscular contraction be seen? How many sets of muscle fibers (in different directions) must the worm have to produce the movements seen?

B. Place the worm under a coverglass in water containing powdered carmine and examine the body margins under the middle power of a microscope. Are cilia present, and if so in what regions?

3. Reactions to stimuli. *A. With a worm in a watch glass touch the anterior end gently, and then more vigorously. Do the same at the posterior end. Note the response to each.*

B. Shade one-half of the container from the light and notice where the worm comes to rest. What is its reaction to light?

C. Place a bit of meat or liver or a drop of blood in the water a little apart from the worm and observe carefully for several minutes. Does the animal display any chemical sense? What can you conclude as to the kinds and location of sense organs?

4. External features. *Distinguish:*

Eyespots (2, dorsal on "head" region)
Auricles (2 lateral lobes on "head")
Pigment spots (on dorsal surface)
Proboscis or pharynx (can be protruded from midventral surface)
Mouth (at end of proboscis)
Reproductive pore (sometimes visible behind mouth)

Draw Fig. 1

5. Digestive system and feeding. *Feed a pale specimen* (starved in the laboratory) *on the red eyes of flies or egg yolk colored with carmine. Watch for the action of the proboscis and for movement of food into the digestive tract* (gastro-vascular system). *Distinguish the main divisions and smaller branches of the tract; these may also be seen in stained specimens mounted on slides.* How generally do the branches penetrate the body? How is food carried to various parts of the system? How is it digested and absorbed? What becomes of any undigested residues? Is there an anus? How does the digestive system compare with that of the hydra and of the frog?

Draw Fig. 2

6. Other organ systems. How is respiration performed? *The flickering flame cells in the excretory system may sometimes be seen in pale living specimens compressed between a slide and coverglass when examined under high magnification. The reproductive organs can be seen only in sexually mature worms carefully stained.*

7. Cellular structure. *Study stained cross sections of a planarian* (text, Fig. 16·4*B*) *under high magnification, and distinguish:*

Epidermis (covers body; single layer of cuboidal cells and slender unicellular glands)
Gastrodermis (lines digestive tract; large columnar cells, some containing vacuoles)
Parenchyma (fills spaces within body; composed of cells without definite walls, a syncytium; includes scattered formative cells)
Muscle fibers (longitudinal, circular, dorso-ventral)
Rhabdites (minute rod-like structures in epidermis, produced by rhabdite cells in outer part of parenchyma)
Nerve cords (2; ventral, inside epidermis)
Reproductive system (various parts, only in adult worms)

How does the planarian compare with the hydra and frog as to the degree of cell specialization in the epidermis, digestive tract, muscles, and nervous system? As to the development of organs?

Draw Fig. 3

8. Regeneration. *A. Each student, or a group, or the instructor, should cut planarians in any or all of the following ways:*

I. Transversely between the eyes and pharynx.

II. Lengthwise in the mid-line.

III. By removing a V-shaped piece behind an eyespot (then separating the cut edges every day or two thereafter to prevent them from growing together).

Place a worm on a clean blotter with a little water. When extended (contracted for "II") *make the cut with one quick stroke of a clean scalpel. Put each piece in a small glass container with pond water and green algae; label each dish with your name, date, and the type of cut made. Examine and sketch each specimen daily or at each subsequent laboratory period until the experiments end. Make outline sketches to show the progress of regeneration, stippling the regenerated parts. Later add water as needed to replace evaporation.*

Begin to draw Fig. 4

B. *For each kind of regenerating fragment, write answers for the following questions, separately for experiments* I, II, *and* III:

1. Does each fragment eventually assume normal form?
2. Which regains normal shape most rapidly?
3. How do fully regenerated worms differ from mature uninjured ones as to size?
4. Does the anterior end become normal in shape?
5. Do two eyes of equal size develop in each piece that lacked eyes originally?
6. Is a pharynx produced in each regenerated worm?
7. What types of cellular changes must occur to bring about regeneration?

8. How does such regeneration differ from the healing of wounds in mankind or higher animals?

9. Other Turbellaria. *Examine any other species of flatworms that may be demonstrated.* To what features do the names PLATYHELMINTHES, TURBELLARIA, TRICLADIDA, and POLYCLADIDA refer?

DRAWINGS

Fig. 1. Entire normal planarian (about 100 mm. long), dorsal view; label parts actually seen.

Fig. 2. Series of outline sketches (25 mm. long) to show the form of body when expanded, contracted, feeding, or in other activities.

Fig. 3. Cross section of body (about 100 mm. wide); outline principal structures identified and fill in details of the cells in a narrow strip from the digestive cavity to the exterior; label to indicate the name and function of each part or kind of cell.

Fig. 4. Series of outline sketches in vertical columns to show condition of each regenerating fragment on the day when cut and on successive days throughout the experiment. Stipple regenerated parts.

Date	Transverse cut (I)		Longitudinal cut (II)		Oblique cut (III)
	Anterior part	Posterior part	Left half	Right half	

EXERCISE 30. FLUKE

Phylum **PLATYHELMINTHES** Class *TREMATODA*

(Storer, "General Zoology," pp. 332–336)

The small flatworms known as flukes are all either external or internal parasites of other animals, chiefly vertebrates. Many of them (Order DIGENEA) live as larvae in one (intermediate) host species and as adults in another species. Larvae of *Clinostomum* occur in cysts in the peritoneum of frogs and fresh-water fishes; adults of *Pneumonoeces* inhabit the lungs of frogs and those of *Gorgoderina* the bladder. The liver fluke of sheep (*Fasciola hepatica;* text, Figs. 16·6, 16·7) has its larval stages in certain fresh-water snails, and the adults live in the bile duct of sheep. Both larval and adult flukes must be fixed, stained, cleared, and prepared as whole mounts for satisfactory study.

1. Adult fluke. *Study a stained specimen under medium and high magnification; distinguish:*

Oral sucker and mouth (at anterior end)
Ventral sucker or acetabulum (posterior to mouth)
Digestive system (mouth, muscular pharynx, short esophagus, "intestine" of two main trunks with many blind-ended branches or caeca)
Excretory duct (clear, in mid-line)

MALE REPRODUCTIVE SYSTEM

Testes (2; much-branched, through much of body except anteriorly)
Vasa deferentia (2; narrow ducts along either side of mid-line)
Seminal vesicle (1; receives the 2 vasa deferentia; near ventral sucker)
Penis (just within genital opening, anterior to seminal vesicle)

FEMALE REPRODUCTIVE SYSTEM

Ovary (1; branched; on right side in anterior third of body)
Oviduct (connects ovary to oötype)
Yolk glands (many, minute, brown, along sides of body)
Yolk ducts (2; along sides of body, connect to oötype)
Oötype (median, surrounded by Mehlis or shell gland)
Uterus (from oötype to genital opening; large, convoluted, contains many eggs)

Draw Fig. 1

Details of the muscles, excretory organs, nervous system, and epidermis with cuticle are seen best in microscopic sections.

2. Larval stages. *If available, examine larval stages* (miracidium, redia, sporocyst, cercaria) *of Fasciola or other flukes.*

Draw Fig. 2

What features of structure, physiology, and natural history in a fluke are different from those of a planarian? How do these differences relate to the life of the fluke as a parasite in another animal? Why is the reproductive system proportionately so extensive? What conditions are necessary for successful continuance of the sheep fluke as a species? Is infection by this fluke of economic importance? How may infection be prevented? What are some flukes that parasitize mankind?

DRAWINGS

Fig. 1. Fluke (125 mm. long), dorsal view; draw and label parts identified. Show only small portions of the intestine, yolk glands, and testes.

Fig. 2. Larval stages of a fluke: miracidium, redia, or cercaria (each 25 mm. long).

EXERCISE 31. TAPEWORM

Phylum **PLATYHELMINTHES** Class *CESTOIDEA*

(Storer, "General Zoology," pp. 336–340)

The tapeworms are all internal parasites. The adult lives in the intestine of some vertebrate (final host) and the larva in the tissues of another species of animal (intermediate host) upon which the final host commonly feeds (text, pp. 339–340). The intermediate and final hosts of some well-known species are: beef tapeworm (*Taenia saginata*), in cattle and man; pork tapeworm (*T. solium*), in pigs and man; dog and cat tapeworm (*T. pisiformis*), in rabbits and cats or dogs; dog tapeworm (*Dipylidium caninum*), in fleas or lice and cats or dogs. The adults of different species are 3 mm. to 12 m. (40 ft.) long.

Tapeworms are obtained either by opening and searching the intestines of the final host or from the feces of a host treated with a "vermifuge" to eliminate the worms. The worms may be examined alive or preserved in formalin. Detailed study of their internal structure requires that parts or small entire tapeworms be flattened, fixed, stained, and mounted on slides. The larval stages are dissected out of the organs in which they occur, then fixed and stained for whole mounts. Eggs usually are obtained from the feces of infected hosts; searching for eggs is the common means of determining tapeworm infections in host animals.

1. General structure. The tapeworm body comprises (*a*) a minute knob-like ***scolex*** ("head") with 4 (or 2) cup-shaped ***suckers,*** and in many species, a terminal ***rostellum,*** with ***hooks;*** (*b*) a narrow ("neck") behind the scolex that connects to (*c*) the main body or ***strobila*** consisting of a series of false segments or ***proglottids.*** How many proglottids are present in a complete worm of the species being studied?

Draw Fig. 1

2. Young proglottid. *In a stained whole mount, under medium or high magnification, identify:*

Muscle fibers (minute, scattered)
Excretory canals (longitudinal and transverse)
Longitudinal nerves (slender)
Genital pore (at side of proglottid)

MALE REPRODUCTIVE SYSTEM

Testes (many, scattered, dot-like)
Vasa efferentia (many, very fine)
Vas deferens (1, twisted)
Penis (in pouch)
Cirrus pouch (joins genital pore)

FEMALE REPRODUCTIVE SYSTEM

Ovaries (2; with branched lobes)
Oötype or shell gland
Oviducts (short, to oötype)
Yolk or vitelline glands (at end of proglottid)
Yolk duct (short, to oötype)
Uterus (from oötype; later branched)
Seminal receptacle (small)
Vagina (narrow; from oötype to genital pore)

Transverse sections will aid in identifying some of these structures and in determining their relative positions.

Draw Fig. 2

3. Gravid or mature proglottid. *In a stained specimen find the much-branched uterus, occupying much of the proglottid and containing great numbers of eggs (or embryos).* Most other organs have disappeared.

Draw Fig. 3

4. Larva. *Examine a fresh, preserved, or stained whole mount of the tapeworm larval stage* (cysticercus or bladder worm) *under low magnification. Identify the bladder or cyst, neck, and scolex.*

5. Eggs. *Examine a fresh preparation or permanent mount of tape worm eggs.* Each ***egg shell*** (produced by the shell gland) contains a fertilized ***egg*** or zygote with several ***yolk cells*** and develops into a ***6-hooked embryo.*** How many eggs are produced in a proglottid? In an entire worm during its life?

Draw Fig. 4

What common characteristics of flatworms are seen in tapeworms? What particular adaptations do tapeworms show to parasitic life? Is the tapeworm specialized or degenerate, and why?

How does a tapeworm attach to its host? What is its nourishment? How is this obtained? How is oxygen for respiration obtained? Why are the reproductive systems so well developed?

What is the life cycle of a tapeworm?

What is the effect on a host of a heavy tapeworm infection? How is infection diagnosed? How cured? How prevented?

DRAWINGS

Fig. 1. Complete scolex (50 mm. in diameter). Label the suckers; also rostellum and hooks if present.

Fig. 2. One young proglottid (75 mm. long), showing the reproductive systems. Label all parts seen.

Fig. 3. An old or gravid proglottid (40 mm. long); outline the uterus and show a few eggs.

Fig. 4. Cysticercus (40 mm. in diameter). Label scolex, suckers, cyst wall.

Table 1. List the organ systems of the adult tapeworm and for each describe how it has been modified for successful parasitic life.

EXERCISE 32. ROUNDWORM

Phylum **NEMATHELMINTHES** Class *NEMATODA*

(Storer, "General Zoology," pp. 343–354)

The nematodes are unsegmented worms of slender form; some are of microscopic size and others up to 300 mm. and more in length. Many species are free living in soil and fresh waters, and many others are parasites in the tissues and fluids of plants and animals. Examination of bottom materials and dense plant growths from quiet, fresh, or foul waters under a microscope usually will reveal some roundworms, and others can be found in damp soils. Search of the intestines, lungs, bladder, and other organs of domestic or wild animals often will yield examples of parasitic species (text, p. 349) of various sizes. Eggs of parasitic species are found by microscopic examination of the feces of infected hosts. Entire specimens are preserved in 5 per cent formalin or 70 to 80 per cent alcohol, and the eggs in these or other fluids.

The common intestinal roundworm of the pig (*Ascaris lumbricoides*) serves well for dissection, as does that of the horse.

1. External features. *Specimens should be washed well in water, both before and after being opened, because the body vapors are irritating to mucous membranes of the human nose and eyes. Identify:*

Cuticle (thin, covers entire body)
Mouth (anterior, between lips)
Lips (3, small, spherical)
Anus (at posterior end)
Longitudinal lines (dorsal, ventral, lateral)
Penial setae (in male)
or
Genital pore (in female)

Draw Fig. 1

What type of symmetry is present? Of what service is the cuticle (compare planaria and tapeworm)?

2. Internal structure. *Pin the specimen, dorsal side up, under water in a dissecting pan. With scissors, cut the body wall the entire length near the dorsal line, using care not to damage any internal organs; then pin out the body wall. Identify:*

Digestive tract (muscular pharynx, stomach-intestine, rectum)
Muscles (in body wall)
Pseudocoel (space between body wall and internal organs)
Male reproductive system (testis, vas deferens, seminal vesicle, ejaculatory duct)
or
Female reproductive system (2 ovaries, oviducts, and uteri; single vagina and genital pore)

Draw Fig. 2

What is the food? How is it obtained? How is the structure of the digestive tract related to the kind and availability of food? How does food digested within the worm reach other parts of its body? How does the pseudocoel of a nematode differ from the coelom of a frog?

3. Cross section. *Study a stained cross section of Ascaris or other nematode under the compound microscope. Identify:*

Cuticle
Epidermis (cell walls present?)
Muscle cells (beneath epidermis)
Pseudocoel (space between body wall and internal organs)
Digestive tract
Body lines (dorsal, ventral, 2 lateral)
Excretory canals (in lateral lines)
Nerve cords (in dorsal and ventral lines)
Male sex cells (spermatogonia, spermatocytes, spermatozoa; in sex ducts)
or
Female sex cells (ova in oviducts; developing ova with shells, in uterus)

Draw Fig. 3

4. Living nematode (free-living or parasitic). *Examine specimens if available.* What types of body movements are possible? How are these related to the placement of the muscle fibers in the worm's body? How do these movements compare with those possible in a flatworm?

5. Parasitic nematodes. *Study examples of hookworm, trichina worm, or other parasitic species that may be demonstrated, including eggs.*

What are some major differences between roundworms and flatworms? What is the economic importance of root nematodes? How do they become distributed? In what host animals may trichina worms occur? How does mankind become infected with the trichina worm? How may he avoid infection?

In what region of the United States is infection with hookworm prevalent? What is the effect of severe hookworm infection? How does mankind become infected with hookworm? How may an infection be cured? How may infection be avoided? Are all nematodes harmful?

DRAWINGS

Fig. 1. Entire *Ascaris* (150 mm. long), lateral view; show and label external features identified.

Fig. 2. Dissected *Ascaris* (150 mm. long), dorsal view. Move digestive tract to one side and spread reproductive organs as necessary to show all parts clearly; label fully.

Fig. 3. Transverse section of nematode (75 mm. in diameter). Outline the layers of the body wall and the internal organs present, then draw a few cells of each part in detail. Label.

EXERCISE 33. STARFISH

Phylum **ECHINODERMATA** Class *ASTEROIDEA*

(Storer, "General Zoology," pp. 375–382)

The Phylum ECHINODERMATA includes the starfishes or sea stars, brittle stars, sea urchins, sea lilies, and sea cucumbers. All but the last have a limy internal skeleton and hard external spines or plates. They are fixed or slow-moving inhabitants of the

sea, from the high-tide line to considerable depths, and often abundant, but none forms colonies. Species of shallow water are easily collected by hand at low tide, and deeper ones by dredging. Those with skeletons are easily preserved merely by drying, but specimens for dissection are preserved in formalin or alcohol. Eggs of starfishes and sea urchins can readily be obtained in quantity and fertilized as needed; hence they serve for study of early stages in embryonic development and in many experimental researches on animal eggs.

Common species of starfishes used for class work are *Asterias forbesi* and *A. vulgaris* of the Atlantic coast and *Pisaster ochraceus* of the Pacific coast.

1. External features. *A. Study a fluid-preserved specimen in a pan of water, and identify:*

Arms or rays (projecting from disc)
Central disc (poorly defined)
Oral surface (usually concave)
Aboral surface (exposed in life)
Madreporite or sieve plate (small white circular area, off-center on aboral surface of disc)
Anus (minute, centered aborally on disc)
Bivium (the two arms closest to the madreporite)
Trivium (the remaining arms)
Spines (many, short, rough, limy, in patterns over aboral surface)
Mouth (centered in oral surface)
Oral spines (elongate, about mouth)
Ambulacral grooves (one along oral surface of each ray)
Ambulacral spines (slender rods, on margins of ambulacral grooves)
Tube feet (soft, slender, with expanded tips; 2 or 4 rows in each groove)
Eyespot (small, pigmented, on end of each arm)
Tentacle (soft, on end of each arm)

Begin Fig. 1

B. Examine a small area on the aboral surface under a binocular microscope and distinguish:

Papulae or dermal branchiae (thin hollow soft projections between the spines; function as gills)
Pedicellariae (minute pincers with two jaws; in circles around spines and elsewhere)

What is the main axis of the body in relation to the position of the mouth? What type of symmetry is shown? For what type of life is such symmetry best adapted?

2. Pedicellariae. *Scrape a little of the aboral surface with a scalpel, mount the scrapings in water on a slide under a coverglass, and examine under medium magnification. Find the pincer-like pedicellariae with jaws, stalk, and muscles.* How many kinds are present? What function do they serve and how is this accomplished? Why is this function necessary?

Draw Fig. 2

3. Internal structure. *With the starfish in water and the aboral surface uppermost, use stout scissors to cut off the extreme tip of each arm of the trivium. Then cut along the sides of these three arms. Use care not to*

injure any internal organs. In turn lift and carefully remove the aboral surface of each arm, loosening the delicate mesenteries beneath by which the soft organs are attached. Also cut around the disc (but not into the bivium) *and remove the aboral surface, leaving the madreporite in place. Finally cut transversely, at mid-length, through one arm of the bivium to provide a cross section. Identify:*

Coelom or body cavity (space containing internal organs; lined with thin ciliated peritoneum)
Stomach (in disc, thin, sac-like and 5-lobed; cardiac portion, larger, with pleated walls and retractor muscles; pyloric portion, aboral, smaller, 5-sided, and smoother)
Intestine (very slender, short, from pyloric stomach to anus)
Hepatic caeca (a pair in each arm, greenish, long, of many finger-like lobes, each caecum with duct to pyloric stomach; also termed digestive glands, liver, or pyloric caeca)
Rectal caeca (2, small short lobes, at base of intestine)
Gonads (in each arm, below hepatic caeca; bilobed; each attached by duct opening aborally; sexes separate)

Complete Fig. 1

How does each part of the digestive system serve in the digestive process? How is the stomach adapted for taking food of large size? Why are the hepatic caeca so large? How are the functions of circulation, respiration, and excretion performed in the starfish?

Draw Figs. 3 and 4

4. Water vascular system. *Remove the side of the stomach near the madreporite and from the latter trace the parts of this system. If available, examine a demonstration specimen having the system injected with colored mass. Identify:*

Stone canal (limy tube in angle of bivium, from madreporite to ring canal)
Ring canal (hard, circular, around mouth region)
Tiedemann bodies (9, small swellings on ring canal)
Radial canal (from ring canal along each arm [see cross section]; connects by transverse canals to ampullae)
Ampullae (many, small, spherical, in floor of coelom; connect to tube feet)
Tube feet

What is the mode of action of the water vascular system? How do the ampullae and tube feet act to effect locomotion? How do the tube feet serve in food taking? In adhering to solid objects?

5. Skeleton. *If available, examine a specimen from which the soft parts have been removed to show the many limy ossicles of various kinds that form the internal skeleton.* In life these are connected and moved by muscle fibers, whereby the arms can be bent and twisted.

DRAWINGS

Fig. 1. Starfish (175 mm. across), in outline only. Locate the madreporite and anus. In the disc area show parts of the digestive tract.

A. In one arm of the bivium show a little detail of the aboral surface.

B. In the second show the oral surface with ambulacral groove, ossicles, tube feet, tentacle, and eyespot.

C. In one arm of the trivium show the hepatic caeca in place.

D. In a second arm show the gonads.

E. In the fifth arm show a few ampullae and ambulacral plates.

Fig. 2. Pedicellariae (30 mm. long); show two or more types; label jaws and stalk.

Fig. 3. Cross section of arm (70 mm. in diameter); draw from the dissected specimen or a prepared slide. With the latter show the radial nerve and blood sinus.

Fig. 4. Diagram a vertical section (100 mm. long) through one arm and the disc to show the oral-aboral relations of the internal organs.

EXERCISE 34. LAND SNAIL

Phylum **MOLLUSCA** Class *GASTROPODA*

(Storer, "General Zoology," pp. 394–397)

Most mollusks of the Class GASTROPODA have a fleshy head, a ventral muscular foot, and a dorsal visceral mass, the last covered by a membraneous mantle and enclosed in a limy shell of one piece. The shell is coiled in a high spiral on most snails, the spiral is a flattened oval on abalones, and the conical shell of limpets shows no signs of coiling in the adults. Slugs have either a small flat shell within the body, or none. In most snails the soft body may be withdrawn completely into the shell, and some have a disc-like operculum to close the shell aperture. Gastropods live variously in the sea, in fresh waters, or on the land; most terrestrial species inhabit moist environments.

Gastropods of many kinds may be collected easily by hand; those in deep waters are obtained by dredging. Living examples of certain species may be kept in the laboratory, in an aquarium or a terrarium according to their habits, and provided with suitable food. For specimens, if only the shells are wanted, these are dried after the fleshy parts have been removed by immersing the animals in hot water. Entire snails, either land or fresh-water, may be killed with the soft body expanded by immersing them for some hours in water which previously has been boiled (to remove the oxygen) and cooled; such specimens are preserved in alcohol or formalin, but the latter will eventually dissolve out the limy salts of the shell in delicate specimens. The minute larvae of marine mollusks are collected in tow nets of fine-meshed silk drawn through the water. The large masses of gelatinous eggs from many species are deposited on objects in the water; those of land snails are hidden in damp pockets in the soil. Some gastropods are viviparous.

The European brown snail (*Helix aspersa*) or some other sizable land snail forms a convenient example for elementary study.

1. The shell. *A.* The single shell or valve of a snail resembles a narrowly conical tube wound in a close spiral. *Examine an entire shell and also one cut along its axis; identify:*

Apex (pointed closed end; oldest part of shell)
Aperture or opening (with outer and inner lips)
Whorls (complete turns; number?)
Suture (line of contact between adjacent whorls)
Lines of growth (on whorls, parallel to edge of aperture)
Columella (central axis between whorls; see in cut shell)

When the whorls, as viewed from the apex, turn clockwise from apex to aperture, a shell is termed ***dextral*** or right-handed; if counterclockwise, it is ***sinistral*** or left-handed. An alternative method is to hold the apex upward and the aperture toward the observer; the aperture is at the right in dextral shells and at the left in sinistral shells.

Draw Fig. 1

B. If available, examine other shells of the same species and of different species. Are they all exactly alike in any one species? How do shells of young and old individuals of a species differ as to size, shape, and number of whorls? As between different species, what are some of the characters of difference in the shells? Does any shell exhibited have an operculum? Does any have a narrow siphonal canal (grooved prolongation of shell) extending ventrally from the aperture?

C. In a cut or broken specimen, identify the three layers of the shell:

Periostracum (outermost; colored) Prismatic (middle; thickest) Nacre (innermost; glistening)

2. External features. *In an extended snail, preserved or living, identify:*

Foot (ventral; long, broad, flat, and muscular)
Head (anterior; not distinct from foot)
Visceral mass (dorsal; behind head and above foot; enclosed in shell)
Tentacles (2 pairs; on head; slender, often retracted [involuted] in preserved snails)
Eyes (2; on ends of posterior tentacles)
Collar or mantle margin (soft thick roll at edge of shell aperture)
Mouth (ventral on head, below first pair of tentacles)
Pedal mucous gland (opens ventral and posterior to mouth)
Respiratory pore (on collar, above or medial to anus)
Anus (on right side of collar)
Genital pore (minute; on right side of head below tentacles)

3. Internal anatomy. *Gently turn the shell in a counterclockwise direction to remove it from the soft visceral mass within. If this is not pos-*

sible, use forceps to pick away the shell in small pieces, beginning at the edge of the aperture; use care not to damage delicate membranes or structures inside.

The visceral mass is covered by a thin membrane, the ***mantle;*** this is the body wall of the visceral mass, and it secretes the shell. The ***mantle fold,*** a distinctive characteristic of mollusks, extends as a fold of the body wall to roof the ***mantle cavity*** within; the latter cavity extends posteriorly and makes a half turn to end on the right side of the visceral mass.

A. *Examine a demonstration dissection. Then insert the point of small scissors through the respiratory pore and cut the mantle fold* (pigmented membrane) *along the dorsal border of the collar; continue the cut to the posterior end of the mantle cavity and then dorsally; turn up the mantle fold to expose the mantle cavity. Identify:*

Pulmonary veins (on inner surface of mantle, converging to auricle; mantle and veins serve in respiration as lung)
Heart (posterior in mantle fold; delicate) consists of
  Auricle (1; dorsal; receives veins)
  Ventricle (1; ventral; pear-shaped; connects to 2 aortas, anterior and posterior)
Pericardial cavity (space around heart; connects by reno-pericardial pore to kidney)
Kidney (brownish or yellowish; extends dorso-ventrally in narrowed part of mantle cavity behind heart; wall thin, with irregular internal folds; duct from kidney extends along left border of rectum)
Rectum or posterior intestine (tubular, along ventral posterior wall of mantle cavity at base of visceral spiral; connects to anus)
Digestive gland or "liver" (large, grayish brown; occupies upper part of visceral spiral)

Begin Fig. 2

B. *Lift the membranous body wall flooring the mantle cavity and slit it in mid-line, both anteriorly and posteriorly; then pin out or cut away the sides of the body. Pick away the thin connective tissues to expose the organs.* From each posterior tentacle there extends a (blackish) ***retractor muscle,*** and similar muscles (or tendons) from several other organs extend to the ***columella muscle*** which is centered in the visceral spiral. What is the function of these retractor muscles? The organs lie within the large perivisceral or ***body cavity,*** which is a hemocoel containing blood. In mollusks the coelom is represented only by the cavities within the pericardium and kidney(s).

4. Reproductive systems. *Helix* is monoecious (hermaphroditic); each snail contains both male and female reproductive systems, which occupy much of the body cavity. *Inside the right-hand body wall* (opposite the genital pore), *find the genital atrium and trace out the reproductive organs:*

Genital atrium (short, rounded; joined to both penis sac [medial] and vagina [at right])

Penis sac (slender, oval; contains short penis)
Flagellum (long, slender folded appendage of penis sac)
Sperm duct (branches off flagellum to run parallel to oviduct)
Vagina (short; walls thick and muscular)
Mucous glands (1 or 2; joined to vagina; each of several finger-like lobes)
Dart sac (large oval bulb, joined to vagina)
Oviduct (slender, wavy; from vagina to albumen and hermaphroditic glands; parallels sperm duct)
Spermathecal duct (long, slender; extends from oviduct to small rounded seminal receptacle [spermatheca] at distal end)
Caecum (slender lateral outgrowth on spermathecal duct)
Ovo-testis or hermaphroditic gland (whitish, rounded, many-lobed; within second whorl of viscera behind columella muscle; connects to ends of oviduct and sperm duct)
Hermaphroditic duct (from ovo-testis along albumen gland to distal ends of oviduct and sperm duct)
Albumen gland (elongate, yellow; on anterior base of visceral whorl; supplies food for eggs)

5. Digestive system. *A. Cut across the genital atrium and gently turn the reproductive organs back over the visceral mass to expose the digestive system. Beginning anteriorly, identify:*

Buccal mass (muscular; around mouth and between anterior tentacles)
Esophagus or pharynx (slender; from mouth posteriorly to crop)
Crop (large, thin-walled, in left side of body cavity)
Salivary glands (thin, yellowish, on outer surface of crop; each with duct to buccal cavity)
Stomach (small, roundish, embedded in liver)
Liver (see Par. 3)
Intestine (slender, tubular, loops in visceral spiral and then returns to right side)
Rectum (continuation of intestine; see Par. 3)
Anus (see Par. 2)

B. Pull the buccal mass forward and cut off its connections to the columella muscle and the esophagus; dorsal in the mouth cavity see the transverse horny brown jaw. Split the buccal mass middorsally and spread open; in the floor of the mouth find the pale-colored horny radula (rasping organ); *examine the latter under magnification to see the minute backward-pointing teeth arranged in rows.*

6. Nervous system. *Dorsal to the pharynx, find the pair of white cerebral ganglia and trace their connections to the ventral pedal ganglia close beneath; the visceral ganglia are located some distance posteriorly.*

Complete Fig. 2

7. The living snail. *A. Examine living land snails or slugs in a moist terrarium. Endeavor to see their methods of locomotion. Watch the action of the tentacles. If possible, see the periodic opening of the respiratory pore.*

If available, watch the activities of fresh-water snails in an aquarium. How is respiration accomplished in most marine snails? In fresh-water snails?

B. *Place a small living snail* (or slug) *on a glass slide and allow it to attach to the surface. Then invert the slide over a shallow dish and examine the animal's ventral surface. See the action of the pedal mucous gland which opens just behind the mouth. Watch for the rhythmic waves of muscular contraction in the foot.* What is the purpose of the mucus or slime track? How does the muscular action of the foot move the animal along?

C. *If possible, observe the actions of a land snail when feeding on some kind of green vegetation.* Of what service are the jaw and radula in feeding?

In the cold of winter, or during dry weather, a land snail withdraws its body into the shell and secretes a plate (epiphragm) of limy materials over the opening. Of what benefit is this practice?

DRAWINGS

Fig. 1. Land snail, body extended (100 mm. long), from right side; show and label parts named in Par. 2.

Fig. 2. Internal structure of land snail (150 mm. long), from left side. Show and label all organs identified in Pars. 3–6. For convenience in drawing, extend reproductive organs above body and visceral whorl.

EXERCISE 35. FRESH-WATER MUSSEL

Phylum **MOLLUSCA** Class *PELECYPODA*

(Storer, "General Zoology," pp. 390–392; 398–405)

Animals of the Phylum MOLLUSCA have soft bodies which, in many species, are enclosed in a limy shell of one or more parts. The clams, mussels, and other bivalves (2-shelled) of the Class PELECYPODA are mostly marine, but certain kinds inhabit fresh waters. Some move freely, others are attached to solid submerged objects, and still others burrow (text, Fig. 20·13). Unlike other mollusks, the bivalves have no head region and no means for rasping or cutting large food; they subsist upon microscopic floating organisms (plankton) and organic debris which are drawn with water between the valves and passed to the mouth.

Mollusks inhabiting shallow waters are collected by hand, and those in deeper waters by dredges (or tongs). If only the shells are to be saved, these are cleaned and dried; entire specimens are preserved in formalin or alcohol. Types used for elementary study include the fresh-water clams or mussels (*Unio*, *Anodonta*, or *Lampsilis*) common in many streams, ponds, and lakes, and also marine clams such as the hard-shell (*Venus*) and soft-shell (*Mya*).

1. External features. *Identify:*

Valves, right and left
Margins: anterior, posterior, dorsal, and ventral
Umbo (dorsal swollen part of each valve)
Lines of growth (concentric)
Siphons, incurrent and excurrent (posterior, fleshy, short)
Margin of mantle (fleshy band just within edges of valves)
Foot (fleshy, can be protruded through antero-ventral margin of mantle)

Draw Fig. 1

What is the oldest part of a valve? If the heavier lines of growth indicate age in years, how old is the specimen?

2. The shell. *A. Examine a demonstration dissection. Then place a clam in water, raise the left valve slightly and insert a scalpel close beneath. Move the blade back and forth to free the sheet-like mantle adhering to the inner surface and also to cut the muscles attached to the valve. Press the ventral margins of the valves together and then release them.* What happens and why?

B. Remove the left valve completely and find:

Hinge ligament (elastic; dark; on dorsal margin)
Hinge teeth (near dorsal margin; none in *Anodonta*)
Pallial line (on inner surface, where margin of mantle attaches)

On the inner surface of the left valve find also the attachment scars of the following muscles:

FUNCTIONS OF MUSCLES

Anterior adductor (large)	*To close valves*	Posterior adductor (large)
Anterior retractor (small)	*To withdraw foot*	Posterior retractor (small)
Anterior protractor (small; behind adductor)	*To extend foot*	

How are the hinge teeth, the hinge ligament, and the adductor ~~muscles~~ interrelated as to function?

Draw Fig. 2

C. Examine the broken edge or a thinly ground section of a valve under low magnification and identify the layers:

Periostracum (outermost, thin, dark; organic in composition)
Prismatic layer (middle, thickest near dorsal margin)
Nacre or mother-of-pearl layer (innermost, smooth, glistening)

What produces each of these layers?

D. Place a small fragment of shell in hydrochloric acid and another in sodium hydroxide solution. Watch the results. Of what is the shell composed? Where are the materials obtained for shell formation?

3. Internal structure. *A. Place the right valve containing the soft body in water and locate the following parts:*

Muscles (5; scars seen on shell; note direction of fibers in each)
Mantle (thin fleshy sheet, of left and right lobes; internal organs enclosed in mantle cavity between lobes)
Siphons[1] (formed of thickened posterior edges of mantle margin)
  Incurrent siphon (ventral, with short sensory papillae inside margin)
  Excurrent siphon (close above preceding; exit for water)

B. Lift the left lobe of the mantle and cut it off, beginning at the incurrent siphon; avoid damaging the soft parts beneath. Find:

Gills (2 pairs, right and left; thin sheets with vertical ribs; cut off left pair along their dorsal margin)
Visceral mass (large, soft; behind anterior adductor muscle)
Foot (ventral; firm muscular projection from visceral mass)
Labial palps (2; thin soft lobes below anterior adductor muscles)

4. Digestive tract. *Carefully scrape or pick away the visceral mass; medially it contains parts of the digestive tract and laterally the two much-branched gonads; locate:*

Mouth (small opening between labial palps)
Esophagus (short tube behind mouth)
Stomach (rounded; dorsal in visceral mass)
Digestive glands or "liver" (2; one on either side of stomach)
Intestine (coiled, in visceral mass; often contains a soft gelatinous rod, the crystallline style, providing a digestive enzyme)
Rectum. (from intestine, posteriorly through heart and then dorsal to posterior adductor muscle)
Anus (enters excurrent siphon behind posterior adductor muscle)

5. Circulatory system. *Dorsally, behind the visceral mass, find the slender thin-walled pericardial sac enclosing the heart. Remove the left wall of the sac and identify:*

Pericardial cavity (space within pericardial sac)
Heart, composed of
  Auricles (2; ventro-lateral; thin-walled)
  Ventricle (1; median; around rectum)
Aortas, anterior and posterior (from ventricle at either end of pericardial sac)

At either side of the pericardial sac, postero-ventrally, is a blackish soft hollow ***kidney***.

Draw Fig. 3

6. Gills. Each gill consists of two soft thin plates (lamellae) composed of many narrow vertical gill bars; the plates are joined internally

[1] In marine bivalves the siphons are of varied form; in the soft-shelled clam (*Mya*) both are contained in a common muscular "neck" that may be greatly extended (text, Fig. 20·13).

by narrow dorso-ventral partitions (interlamellar junctions) that divide the space within the gill into a series of vertical water tubes. Water from the mantle cavity passes in pores between the gill bars (which contain blood vessels; text, Fig. 20·11) into the water tubes, and leaves by a slender cylindrical (suprabranchial) chamber connected to the excurrent siphon. (The water tubes in a female fresh-water clam serve as brood chambers to hold the developing eggs.)

Gently pass a small probe through the excurrent siphon and anteriorly into the suprabranchial chamber of the outer gill; cut away the lateral wall of the chamber to see the ends of the partitions and tubes within. Cut off a small piece of one gill, separate the two plates along one cut edge, and examine in water under a binocular microscope to see the gill bars, with their minute supporting bars and pores (ostia) *between the bars.*

Draw Fig. 4

7. Nervous system. *Remove the soft body from the right valve* (in the same manner as used in separating the left valve) *and turn the viscera right side uppermost. Remove the right mantle, gills, and labial palp, then pick away the tissue below the bases of the palps to find the whitish fused cerebral ganglia. Trace the cerebro-pedal nerve to the pedal ganglion dorsally in the foot; also trace the cerebro-visceral nerve beneath the kidneys to the fused visceral ganglia below the posterior adductor muscle.*

8. Cross sections. *If available, examine gross cross sections made from an entire preserved clam at several levels along the body and identify as many as possible of the structures named in preceding paragraphs.*

Draw Fig. 5

9. Behavior. *Observe one or more living clams in an aquarium with a deep layer of sand under the water; be prepared to answer the following questions.*

A. POSITION. What is the normal position of the animal when at rest? When moving? Which end travels foremost? When laid on its side, how does a clam right itself? How does a clam burrow in the sand?

B. TOUCH. What happens when the edge of the mantle is touched lightly? What part seems to be most sensitive? When the two siphons are touched separately, which appears to be the more sensitive and why?

C. LIGHT. When a strong light (or sunlight) has been shining on the aquarium until a clam has its siphons opened and a shadow then falls on the siphons, what takes place? Of what probable advantage is this reaction in nature?

D. WATER CURRENTS. When water containing powdered carmine or other fine suspended particles is introduced near the incurrent siphon with a pipette, what occurs? Does the carmine later reappear, and if so where? What essential functions are served by these water currents?

Through what structures and spaces does water pass in a clam between the incurrent and excurrent siphons?

10. Reproduction. ***A.*** *Examine a female fresh-water clam that contains developing young in the brood chambers* (some or all water tubes of the gills).

B. *Remove and examine some of the young stages under a binocular microscope;* each is a minute glochidium with two valves and a larval thread between; in *Anodonta* the valves bear ventral hooks.

C. *See also, if available, a fish with glochidia enclosed in superficial capsules on the gills or skin.*

How do the glochidia escape from the parent clam and become attached to the fish? What is the complete life cycle of a fresh-water clam? Of what advantage is the glochidial stage on the fish? How do marine mollusks that have no glochidial stage become distributed?

What are some important economic uses of bivalved mollusks? What are some important species? Whence come "pearl" buttons? How are pearls formed? Are any human diseases carried by bivalves?

11. Classification. *If available, examine a collection of cleaned shells of bivalve mollusks, including:*

Species (several examples of one species to illustrate individual variation)
Genus (representatives of several species to show characters that separate species)
Family (examples of different genera to show shell differences between genera)

The general classification of mollusks is based upon characters of both the shells and soft parts.

DRAWINGS

Fig. 1. Fresh-water clam (75 mm. long), external view, left side.

Fig. 2. Inner surface of left valve (75 mm. long), ventral margin uppermost.

Fig. 3. Internal organs within outline of right valve (125 mm. long); show and label muscles, entire digestive tract, parts of circulatory system, gonads, gills, mantle, and siphons.

Fig. 4. Small part of gill much enlarged. Show gill bars, ostia, and water tubes.

Fig. 5. Cross section of entire clam (125 mm. high), through heart. Show and label all parts identified.

EXERCISE 36. EARTHWORM

Phylum **ANNELIDA** Class *OLIGOCHAETA*

(Storer, "General Zoology," pp. 412–424)

Members of the Phylum ANNELIDA are all worms, most of which have the body divided into a series of similar ring-like segments or ***somites*** and have a definite body cavity or ***coelom*** lined by peritoneum. They also have few to many fine hard spines or ***setae*** projecting from the sides of the body wall that assist in locomotion. The phylum includes many marine species that variously swim, burrow, or live in tubes; the earthworms that inhabit the soil and related forms in fresh waters; the leeches that live in water or on damp soil; and some other types.

Earthworms live in moist loamy soil and burrow extensively. At night they come to or onto the ground surface to feed, mainly on decaying vegetation. They may be collected most easily at night, with the aid of a flashlight, especially after a rain when many are out on the surface. Uninjured worms may be kept alive for weeks in a covered box supplied with old leaves and leaf mold or light loamy soil kept at 60°F. or cooler. Specimens for dissection must be anesthetized (lest they contract severely) and laid out straight for preservation. Small individuals that are to be sectioned for microscopic study are placed on damp paper for some days before being anesthetized and fixed so as to rid the digestive tract of sand grains which otherwise would damage the microtome knife and tear the sections.

The large earthworm (*Lumbricus terrestris*) of eastern United States and Europe is used for dissection, and smaller species for microscopic study. During dissection, specimens must be kept in water to avoid drying and shriveling of organs. Throughout this exercise roman numerals are used to designate the somites, beginning at the anterior end.

1. External features. ***A.*** *Examine an entire worm in water, using a hand lens or binocular microscope, and identify:*

Anterior end
Mouth (in anterior end)
Prostomium (lobe over mouth)
Posterior end
Anus (vertical slit in posterior end)
Dorsal surface (darker, with median blackish line of dorsal vessel)
Ventral surface (slightly flattened)
Somites or segments (ring-like divisions of body)
Clitellum (smooth swelling over several anterior somites)
Setae (minute spines on somites; number? location?)

OPENINGS IN BODY
(besides mouth and anus)

Oviducts (2, small, ventral, on XIV)
Sperm ducts (2, ventral, on XV)
Seminal receptacles (2 pairs, small, lateral, between IX–X and X–XI)
Nephridiopores (2 per somite, small, latero-ventral)
Dorsal pores (minute, middorsal in furrows between somites)

Draw Fig. 1

B. *Count the somites in your specimen* (1) *anterior to the clitellum* (but omit prostomium), (2) *in the clitellum*, (3) *behind the clitellum. Report these to the instructor who will assemble the counts made by members of the class.*

Draw Fig. 2

What type of symmetry is present? Where is the longitudinal axis located? Where is the sagittal plane? To what degree is antero-posterior differentiation shown?

C. CUTICLE. *Strip a little of the thin cuticle covering the exterior surface from* (1) *the anterior end,* (2) *the middle of the body, and* (3) *the posterior end. Float out each in water, spread on a glass slide, drain, and put away to dry for later use* (Par. 6B).

2. Internal structure. *Cut across the specimen about* 25 *mm. behind the clitellum; keep all parts of the worm until the exercise is completed.*

A. *From the posterior part cut a piece of* 2 *or* 3 *somites, and examine both anterior and posterior surfaces under low magnification; remove a slightly longer fragment and cut it dorso-ventrally.* The body is essentially one tube (body wall) surrounding another (digestive tract); the space between (coelom) contains various organs and is divided by transverse partitions (septa). *Determine the relations of these various parts and of the septa to the somites and surface furrows.* The dorsal fold within the intestine is the ***typhlosole.***

B. *Examine a demonstration showing how the dissection of the anterior portion is to be made. Holding this part of your worm with dorsal side uppermost* (recognized by dark line of dorsal vessel) *and using sharp scissors, cut forward through the dorsal body wall* (only), *just to the left of the mid-dorsal line to about somite* IV; *keep the point of the lower scissor blade from damaging internal organs. Pin down the specimen by the posterior end, placing the worm near the edge of the dissecting pan or pad. Beginning posteriorly grasp the cut edges of the body wall with forceps, and use a needle to release the thin transverse septa from their attachments to the digestive tract. Keep the blackish dorsal vessel uppermost; loosen the septa equally on the two sides. As the body wall is spread, fasten it down with pins along the margins; place pins, slanting outward, in each side of somites* XX, XV, X, *and* V *for convenience in referring to organs. Finally cut and spread somites* IV *to* I. *Wash away any coagulated debris around the organs with water from a pipette.*

C. *Locate the following organs:*

DIGESTIVE SYSTEM

Buccal cavity (just behind mouth)
Pharynx (swollen, with external muscle fibers, III–V)
Esophagus (slender, hidden under seminal vesicles, VII–XIV)
Crop (large, spherical, thin-walled, XV–XVI)
Gizzard (large, thick muscular walls, XVII–XVIII)
"Brain" or suprapharyngeal ganglia (2 small white lobes, dorsal in III)
Seminal vesicles (2 pairs, large, soft, one pair bilobed, IX–XIII)
Seminal receptacles (2 pairs, small, spherical, between IX–X and X–XI)
Nephridia (1 pair per somite, small white coiled tubes, latero-ventral)
Dorsal vessel (median, over digestive tract; filled with blackened blood)

Intestine (with paired lateral pouches in each somite, XIX to anus)

"Hearts" or "aortic arches" (5 pairs, lateral to esophagus, partly hidden under thick septa, VII–XI)

Nerve cord (whitish band on ventral wall of coelom)

Begin Fig. 3

D. Cut across the digestive tract between the pharynx and esophagus, then carefully dissect the latter and other digestive organs free from the septa to beyond the gizzard; cut the septa between somites XII *to* XIV *close to the esophagus. Examine the external surface of the tract under water and then split it dorso-ventrally; find three pairs of calciferous glands lateral on the esophagus.*

Complete Fig. 3

What kinds of materials are taken as food? How are these obtained? Is the food of high or low nutritive value? Can the worm separate the good and waste portions? What is the function of each organ in the digestive system? What happens to the food from the time it enters the mouth until it passes out of the digestive system? What are the respective paths of usable food that is digested and of indigestible wastes?

3. Circulatory system. *A. The dorsal blood vessel and hearts have been noted. Find the ventral vessel suspended in a mesentery between the intestine and nerve cord. Then, from the posterior fragment of the worm, remove about* 25 *mm. of intestine and discard; rinse the area with water from a pipette, find the nerve cord, and carefully dissect free a piece of the cord extending through* 2 *or more somites. Mount ventral side up in glycerin-carmine, let stand a few minutes, then add a coverglass and examine under a microscope. Find small blood vessels as follows:*

Subneural vessel (ventral to nerve cord)
Latero-neurals (one at either side of nerve cord)
Connecting vessels (between subneural and latero-neurals)
Roots of parietal vessels (2 per somite; to body wall, subneurals, etc.)

Examine fragments of the worm (as in Par. 2A) *for other small vessels of the blood system.*

Draw Fig. 4

B. Form a shallow frame of wet paper on a slide, place a small live earthworm within, lay on a second slide, and compress slightly with a rubber band around each end; examine under a hand lens or binocular microscope. Determine the direction of blood flow in the dorsal vessel and hearts; also, if possible, in the ventral vessel.

C. With a capillary pipette or hypodermic syringe, withdraw some coelomic fluid from a live worm (Lumbricus or Tubifex), place on a slide,

add salt solution as needed, and examine at once under high magnification with the compound microscope, using weak illumination. What kinds of cells are present and what are their characteristics? How and where do they circulate?

Draw Fig. 5

D. From these dissections and studies, together with the use of diagrams and information in the text (Table 21·1), *understand the various circulatory paths of blood in the living worm.*

What are the various functions of the circulatory system? Of what is the blood composed? How does the system of an earthworm differ from that in a mollusk? What organs perform the function of respiration in an earthworm? How is the O_2–CO_2 exchange accomplished for tissues in the intestinal wall?

4. Excretory system. *A. In the region behind the clitellum carefully remove the intestine* (if not previously done), *separating it carefully from each septum in turn with a dissecting needle. Rinse with water from a pipette and examine the field under low magnification to find the whitish nephridia* (2 per somite).

As directed by the instructor, these may either be studied in place or one may be dissected free, mounted entire on a slide in a drop of water, and examined under low and medium magnification to find:

Nephridial funnel or nephrostome (small ciliated disc on anterior surface of septum close to nerve cord)
Stalk (slender, from funnel through septum)
Secreting tubule (convoluted; supplied with fine blood vessels)
"Bladder" (enlarged, muscular; often contains parasitic nematodes)
Nephridiopore (connection of bladder through body wall to exterior)

Draw Fig. 6

B. Remove a nephrostome separately by cutting with fine scissors the septum at either side and the stalk behind; mount in water on a slide under a coverglass and examine under high magnification with the compound microscope. Identify the following cells, all ciliated:

Central guard cell (large, nucleus central)
Marginal cells (many, around central cell)
Stalk cells (cilia directed into cavity of tube)

If living worms are available, examine a nephridium freshly removed to see movements of the cilia.

Draw Fig. 7

What substances are removed by the nephridia? Where are these drawn from? What organs in the frog or man perform equivalent functions? What are the differences between excretory wastes and feces?

5. Reproductive system. *A. Wash the anterior part of the worm free of debris. With the needle and forceps, pick away the soft seminal vesicles on one* (left) *side to reveal the testes and sperm funnels. Wash out the loose masses of sperm in the funnels. Under low magnification identify:*

MALE REPRODUCTIVE SYSTEM

Testes (2 pairs; minute, antero-ventral at base of vesicle, X, XI; often missed)
Sperm funnels (2 pairs; tough, white, much-folded, attached posteriorly, X, XI)
Vasa efferentia (2 pairs; short, kinky; one from posterior end of each funnel)
Vasa deferentia (2; straight, posteriorly on floor of coelom, to opening in XV)

FEMALE REPRODUCTIVE SYSTEM

Ovaries (2; near nerve cord on septum between XII–XIII)
Ovarian funnels (2; each opens toward an ovary; in septum between XIII–XIV)
Egg sacs (2; often present; on sides of funnels in XIV)
Oviducts (2; one from each funnel to external opening on XIV)
Seminal receptacles (2 pairs; rounded, whitish, between IX–X and X–XI)

B. Remove some material from the interior of a seminal vesicle, tease out in water on a slide, lay on a coverglass, and examine under medium magnification. Likewise remove an ovary, and find ova (egg cells) *in various stages of development.*

Draw Fig. 8

C. By study of the text, learn how and where the male and female sex cells are produced, how the spermatozoa complete development in the seminal vesicles, and how the sex cells travel to the exterior. Likewise study the mating process in which sperm are exchanged between two worms and the subsequent production of fertilized eggs and the cocoons that enclose them. Understand how each part of the two reproductive systems functions in all these processes of reproduction.

6. Nervous system. *A. Carefully remove any parts of the reproductive system that obscure the nerve cord in the anterior somites; also lift and cut off the pharynx, using care to preserve nerve structures beneath. Identify:*

Brain or suprapharyngeal ganglia (2 lobes, joined; dorsal in III)
Connectives (2; around sides of pharynx)
Subpharyngeal ganglia (2, fused; below pharynx in IV)
Ventral nerve cord (median along floor of coelom)
Ganglia (slight enlargement of cord in each somite)
Segmental nerves (paired, extending laterally in each somite; number per somite?)
Anterior nerves (from "brain," connectives, etc.)

Draw Fig. 9

B. Examine under high magnification the samples of cuticle previously prepared (Par. 1C), *and find:*

Mucous pores (many; separate; open over gland cells in epidermis)
Sensory patches (small round or oval areas, each with several openings formerly over groups of sensory cells)

The much larger openings into seta sacs and nephridiopores also may show.

Draw Fig. 10

What are the relative numbers of sensory patches present on the anterior end, posterior end, and mid-body somites, respectively? How may this distribution of sensory structures be related to the habits of a living earthworm?

7. Microscopic structure. ***A.*** CROSS SECTION. *Examine, under low magnification, a thin stained cross section through an entire earthworm* (behind the clitellum); *move it about to find the various parts. Then study cellular details under high magnification. Identify:*

BODY WALL

Cuticle (thin, noncellular, secreted by epidermis)
Epidermis (columnar cells; some oval and glandular, a few sensory)
Circular muscles (thin layer; fibers in longisection)
Longitudinal muscles (thick layer; feather-like pattern; fibers in cross section)
Peritoneum (very thin; of squamous epithelium)

BLOOD VESSELS

Dorsal
Ventral
Subneural
Latero-neural (2)

INTESTINAL WALL

Chloragogen cells (columnar, granular; = modified visceral peritoneum)
Muscle fibers (few, circular and longitudinal)
Blood capillaries and sinuses
Mucosa or lining of intestine (columnar, ciliated cells; some gland cells)
Typhlosole (dorsal fold within intestine)

NERVE CORD

Sheath (connective tissue, with blood vessels and muscle fibers)
Giant fibers (3, dorsal, large, clear)
Nerve cells (dark-stained) and their processes (in ganglion)
Nerve fibers (many, seen in cross section)

Draw Fig. 11

Some sections show parts of the setae and their muscles. Confusing parts of the nephridia and septa show in the coelom; scattered groups of free amoebocytes, also may be seen. The typhlosole is filled with chloragogen cells. Blood vessels, nerves, and connective tissue occur among the muscles, which are nonstriated. What service does each of these perform? What are the respective functions of the two muscle layers of the body-wall? What are the presumed functions of the chloragogen cells? How is food moved along the intestine?

B. PARASAGITTAL SECTIONS. *Study a series of selected parasagittal sections. These are cut parallel to the median sagittal plane* (thus at right angles to the cross section) *and will show the antero-posterior and dorso-ventral relations of various organs from the median plane to near the lateral*

body wall. Not all the structures listed below will appear in any individual section.

Besides the structures listed under Par. 7A, identify the following:

Septa
Hearts
Peritoneum (covering various organs)
Blood vessels in various organs

Digestive Tract

Prostomium
Peristomium (somite I)
Mouth
Pharynx and muscles
Esophagus
Calciferous glands
Crop
Gizzard
Intestine

Nervous System

Suprapharyngeal ganglion
Subpharyngeal ganglion
Anterior nerves
Ventral nerve cord

Male Reproductive System

Testes (with clumps of small spermatogonia)
Sperm funnels (ciliated; often contain mature sperm)
Seminal vesicles (containing clumps of maturing sperm; also brownish parasitic protozoans, *Monocystis*)
Vasa efferentia and vas deferens

Female Reproductive System

Ovary (containing small oögonia and larger oöcytes or eggs)
Egg funnel (ciliated)

Draw Fig. 12

8. Behavior. *Wash a dissecting pan or glass plate so that no preserving fluid is present, and cover with wet paper, on which place an active live earthworm. Handle the worm gently. For each experiment* (B to F) *use a fresh worm, and in each apply the stimulus in turn at the anterior end, the posterior end, and the middle of the body.*

A. Movements and locomotion. *Observe carefully how the worm moves and travels.* What changes take place in the size and form of the body during these activities? What anatomical structures in the body produce the changes? Is any rhythm or coordination shown in the movements? *With a hand lens or binocular microscope determine the position of the setae on any one part of the body during contraction and also during elongation.* How do the setae aid in locomotion?

Turn the worm upside down and study its righting movements. Are these always the same? Does it turn in one direction more than another?

Slant the surface on which the worm rests or place the animal on an inclined damp rough cloth or towel. Can it climb? If so how is this accomplished?

B. Contact. *With the point of a needle, touch lightly in turn different body parts.* How does the worm react? Are there differences in response between the various regions? With repeated stimulation of one region is there evidence of fatigue?

C. LIGHT. *Using a card with a small* (2-mm.) *hole in the center, allow a spot of sunlight to shine in turn on different parts of the worm.* Is the reaction positive or negative? Is the response the same for all parts of the body? Explain.

D. HEAT. *With a hand lens, focus* (concentrate) *sunlight successively on various body parts.* What response occurs? Is it the same for all regions of the body?

E. MOISTURE. *Observe a demonstration in which a broad glass dish is lined with paper, dampened in one half and dry in the other, and in which worms are placed at random over the entire area.* After an hour or more where are the worms? To what stimulus do the worms react?

F. CHEMICAL. *Moisten the tip of a glass rod with either clove oil or xylol and bring close to* (but do not touch) *different body parts in turn.* What are the results?

Prepare Table 1

9. Other annelids. *Examine, if available, specimens of other representatives of the* Phylum ANNELIDA. What are the general characteristics of the Phylum? What characteristics distinguish the Classes OLIGOCHAETA, POLYCHAETA, and HIRUDINEA from one another?

What are some of the interrelations between the various annelids of each Class and other organisms in their respective environments? Are any species of annelids either useful or harmful to man and if so how?

DRAWINGS

Fig. 1. Earthworm, from anterior end to just behind clitellum (125 mm. long), ventral view; show and label structures and openings named in Par. 1.

Fig. 2. Graph on coordinate paper, showing variations in number of somites before, in, and behind the clitellum, respectively, of all earthworms used by class. Plot numbers of somites as abscissae (horizontal axis) and number of specimens as ordinates (vertical axis).

Fig. 3. Internal organs of anterior 20 somites (125 mm. long), dorsal view; show and label parts listed in Par. 2C.

Fig. 4. Blood vessels adjacent to nerve cord (50 mm. long), ventral view.

Fig. 5. Cells in coelomic fluid (each about 25 mm. in diameter).

Fig. 6. Two somites laid open (125 mm. wide), showing one nephridium in place; label parts named in Par. 4A.

Fig. 7. One nephrostome (50 mm. in diameter) and part of stalk.

Fig. 8. Entire reproductive system (100 mm. long), in outline of somites IX to XV.

Fig. 9. Nervous system of anterior 7 somites (100 mm. long); outline somites.

Fig. 10. Sensory pores and mucous pores in relative distribution on (*a*) an anterior somite, and (*b*) a mid-body somite; much enlarged.

Fig. 11. Cross section of earthworm (125 mm. in diameter); outline the organs and tissue layers; then fill in a few cells of each in a sector from the intestinal cavity through the nerve cord to the cuticle.

Fig. 12. Parasagittal section of anterior 16 somites (200 mm. long); use low magnification while outlining the drawing; number the somites; outline the reproductive organs and show beside each a few of its types of cells.

TABLE 1.—REPORT OF EXPERIMENTS ON LIVING EARTHWORMS

	Stimulus	Reaction or tropism involved	Response (positive or negative)	Degree of sensitivity
Contact	(1) Anterior end			
	(2) Posterior end			
	(3) Middle of body			
	etc.			

EXERCISE 37. CLAMWORM

Phylum **ANNELIDA** Class *POLYCHAETA*

(Storer, "General Zoology," pp. 424–428)

Worms of the Class POLYCHAETA are considered to be somewhat more primitive in basic structure than those of the Class to which the earthworm belongs. There are, however, various specialized types among the former. A typical polychaete has (1) a differentiated head with sense organs, (2) each somite with two lateral fleshy lobes (parapodia) bearing many setae, (3) the sexes usually separate and without permanent gonads, and (4) a microscopic larval stage (trochophore) that swims or floats in the water before settling to the bottom and becoming a minute segmented worm.

All but a few of the polychaetes are marine, and many kinds inhabit seacoasts; *Nereis*, the clamworm, is a common representative, of which different species are 100 to 300 mm. or more in length. By day the clamworm lives in a self-constructed burrow in mud or sand with only its head exposed. At night it may creep on the surface or swim in search of food.

Specimens are dug from their burrows at low tide, anesthetized to ensure that organs of the head region will remain protruded, and then preserved in alcohol or formalin. Eggs and sperm dissected from mature living adults are much used for experimental studies of fertilization and embryology. The trochophore larvae are collected by towing nets of fine-meshed silk through the water at the appropriate season.

1. External features. *Place an entire Nereis in water in a dissecting pan; under low magnification identify:*

HEAD

Prostomium (dorsal, anterior, squarish), with prostomial tentacles (1 pair; medial, small) prostomial palps (1 pair; lateral, conical, short) eyes (2 pairs; dorsal, small, dark)
Peristomium (= somite I) peristomial tentacles (4 pairs; lateral, slender) mouth (broad, ventral)

BODY

Somites (many, essentially alike, each with 2 lateral parapodia)

ANAL SOMITE

Anal cirri (1 pair; slender, soft)
Anus

Draw Fig. 1

2. Parapodium. *Cut off one parapodium close to the body, noting which part is dorsal; lay it flat on a slide, add water and a coverglass, and examine under medium magnification, preferably under a dissecting binocular; distinguish:*

Dorsal lobe or notopodium (with large thin vascular gill plate at top)
Ventral lobe or neuropodium
Aciculum (long thin dark bristle, one in each lobe; attaches to muscles inside body and acts as lever to move parapodium)
Setae (many fine bristles; a bundle in each lobe)
Cirri (2; one on each lobe; slender, pointed, soft)

Draw Fig. 2

3. Internal structure. *By two transverse cuts across the posterior part of the body remove a piece comprising 2 or 3 somites; make one cut in a furrow between 2 somites and the other close to the parapodia. Also remove a slightly longer piece and then cut it lengthwise, in the median plane; from one half-fragment carefully dissect out the intestinal wall. Put all these pieces in a watch glass with water and examine the cut surfaces under low magnification; identify:*

Body wall
Intestine
Coelom (divided by transverse septa)
Dorsal vessel
Ventral vessel
Ventral nerve cord
Nephridia (minute, lateral, near acicula)
Acicula (project into body)
Longitudinal muscles (in body wall; also in paired bundles, dorsal and ventral)
Diagonal muscles (to parapodia)

Draw Fig. 3

4. Digestive system. *Insert the pointed blade of scissors into the mouth and cut backward in the median line through the dorsal walls of the body and digestive tract for about 15 somites; spread and pin the walls laterally in a dissecting pan under water and examine under low magnification.* The muscular ***pharynx*** bears 2 lateral horny ***jaws,*** and the

entrance is lined by many small horny ***teeth;*** in life these parts can be protruded to seize food. *On one side tear the septa between the body wall and digestive tract and examine the outer surface of the latter.* Stout muscles serve to protrude and withdraw the pharynx. Posterior to the latter is a short ***esophagus,*** joined by 2 ***digestive glands,*** and followed by the ***stomach-intestine.***

In what ways does *Nereis* differ from the earthworm as to external features? As to internal structure? What does *Nereis* use for food and how is the food obtained? Considering the differences in the environments occupied by these two worms, and in their habits, of what advantage to *Nereis* are the distinct head and its special sensory organs? How do the structural differences in the digestive system relate to the respective food habits of the two?

DRAWINGS

Fig. 1. Head of *Nereis* (50 mm. high), side view.

Fig. 2. One parapodium (50 mm. high); show and label parts listed in Par. 2.

Fig. 3. Cross section of body (50 mm. high); show and label all parts identified.

EXERCISE 38. CRAYFISH

Phylum **ARTHROPODA** Class *CRUSTACEA*

(Storer, "General Zoology," pp. 436–450)

The great Phylum ARTHROPODA includes the crustaceans, insects, spiders, centipedes, millipedes, and their relatives. Collectively they comprise the majority of all known animal species. Typically the body is segmented and comprised of head, thorax, and abdomen. It bears jointed appendages that are differentiated in both structure and function and is enclosed in a more or less hardened exoskeleton containing chitin. The body contains many specialized muscles and has well-developed sense organs; these enable many arthropods to be quite active and exhibit quick responses to stimuli. All but the crustaceans are predominantly terrestrial.

Members of the Class CRUSTACEA include the crayfishes, crabs, shrimps, water fleas, barnacles, and other types. Most of them are aquatic, many are marine, others occur in fresh or alkaline waters, and a few live on land.

Crayfishes (*Astacus, Cambarus*) of different species inhabit fresh-water streams, ponds, and lakes, or burrows in wet fields. They hide by day and come forth at night to feed on dead animals, small living creatures, and plants. They may be captured by hand from their shelters and also by special traps baited with flesh. The lobster (*Homarus*) of salt waters along the Atlantic coast is closely similar to the crayfishes in structure. Specimens of either for dissection are commonly preserved by injecting formalin into the body and then immersing them in the same fluid; the arterial system sometimes is injected with a color mass to aid in tracing the blood vessels. Speci-

mens should be rinsed in water before use and should be returned to the preservative between laboratory periods, as directed by the instructor.

1. External features. *Examine an entire crayfish and identify:*

Cephalothorax (anterior, head + thorax)
Carapace (common rigid dorsal and lateral covering over cephalothorax; crossed by cervical [neck] groove)
Eyes (2; on stalks at sides of head)
Rostrum (median anterior spine, between eyes)
Gill chambers (2; under sides of the carapace [branchiostegite])
Abdomen (posterior, of 6 somites, each covered by dorsal tergum and ventral sternum; with lateral peg-and-socket joints between somites)
Telson (medial terminal plate at posterior end of abdomen)

PAIRED APPENDAGES

Antennules (short, 2-branched, many-jointed)
Antennae (long, slender, flexible, many-jointed)
Mouth parts (5 pairs; see Par. 2)
Chelipeds (stout, with heavy pincers)
Walking legs (4 pairs; slender, on thorax)
Swimmerets (5 pairs; short, small, 2-branched, on abdomen)
Uropods (lateral plates on last abdominal somite)

BODY OPENINGS

Mouth (ventral, on head)
Anus (ventral, on telson)
Excretory openings (2; ventral, in bases of antennae)
Statocysts (2; dorsal, in bases of antennules)
Oviducts (2; in female; bases of 3d legs)
Sperm ducts (2; in male; bases of 5th legs)

Draw Fig. 1

Is the symmetry strictly bilateral? How does the degree of antero-posterior differentiation shown compare with that in the earthworm? What is meant by cephalization? Is it evident in the crayfish? What structures afford protection to the animal? Why must a crayfish molt at intervals? What is the nature of the chitinous covering over joints? What movements are possible between abdominal somites?

2. Paired appendages. ***A.*** *Examine a demonstration dissection; then fasten a piece of white paper along one side of the dissecting pad or pan on which to pin and number the appendages. Remove the lateral part of the carapace on the right side. Beginning at the posterior end, remove in turn each appendage completely by cutting with a scalpel through its attachment to the body. With the walking legs and appendages behind the mouth, take care to bring away the plume-like gills attached to each. As each appendage is removed, pin it in order, ventral side up, on the dissecting pad, and write down its number; have the proximal ends of all uniformly along one side of the pad.*

B. *Analyze each appendage as to its component parts:* protopodite (basal, attached to body); endopodite (medial extension); exopodite (lateral extension). Are all these components present on all appendages? What do the terms "biramous appendage" and "serial homology"

mean? Which appendages are least specialized (*i.e.*, nearest the presumed ancestral form)? Which appendages are most specialized and for what functions? What differences in appendages are there between males and females?

Draw Fig. 2

C. CHELIPED. *From the cheliped already removed, cut off the hardened shell on the outer side of the pincer* (chela) *and also over one or more proximal joints. Open and close the pincer to see the action of the muscles that operate it.* Which is larger and why? Are all the joints on this appendage in the same plane? Why? Of what service is the exoskeleton in relation to muscular movement and locomotion?

Draw Fig. 3

3. Respiratory system. *A. Carefully remove the left side of the carapace over the gills, leaving the latter in place. Working under water, distinguish the types of gills and numbers of each:*

Podobranch (attached to base of an appendage)
Arthrobranch (attached to membrane joining appendage to thorax)
Pleurobranch (attached to thoracic well; none in *Cambarus*)

B. In a large gill (such as podobranch on 3d walking leg), *identify:*

Basal plate Stem Lamina Plume

Draw Fig. 4

C. Find the narrow scoop (scaphognathite) *on the 2d maxilla which draws water through the gill chamber* (see Par. 4).

4. The living crayfish. *A.* MOVEMENTS. *Examine a living crayfish in an aquarium.* How does it walk? What appendages are used? Does the animal right itself when inverted? Can it do so when out of water? How does the crayfish swim? What structural parts serve in this sort of locomotion? Over what parts of the immediate environment can the antennae and the chelae be moved? Where does the crayfish take shelter when undisturbed, and why?

B. RESPIRATION. *Watch a demonstration showing the movement of water* (containing carmine particles) *through a gill chamber.* Where does water enter and leave the chamber? What interchange of gases occurs in the gills? How does their structure facilitate this exchange? Why cannot respiration occur elsewhere on the body?

C. FEEDING. *Observe the feeding of a crayfish. Give particular attention to actions of the antennules, antennae, and appendages about the mouth.*

5. Opening the body. *A. Carefully remove the remaining dorsal part of the carapace with scissors. Insert one blade close beneath the free posterior edge of the carapace and cut forward parallel to the inner wall of the gill*

chamber on either side and across the rostrum anterior to the eyes, leaving the latter in place; use care not to damage soft organs within the thorax. Then cut backward through the lateral part of each abdominal tergum and remove the latter. Use forceps to pick off the pigmented epidermis which lies just beneath the exoskeleton (and secretes the latter).

B. *The thoracic organs in dorsal view, beginning anteriorly, are:*

Stomach (thin-walled)
Mandibular muscles (origin of one at either side of stomach)
Gonads (2; fused posteriorly; ovaries in female, testes in male)
Heart

6. Circulatory system. *Identify and trace out the following so far as possible:*

Pericardial sac (thin-walled; surrounds heart, posterior in thorax)
Heart (several-sided; with 3 pairs of valves or ostia)

ARTERIES

Ophthalmic (1; to eyes and antennules)
Antennary (2; to antennae and mouth region)
Hepatic (2; to digestive glands)
Dorsal abdominal (1; to abdomen)
Sternal (1; from preceding; passes ventrally)
Ventral thoracic, Ventral abdominal } (see Par. 11)

What is the complete path of blood circulation? Are veins present? How and from where does the heart receive blood? How does the circulatory system in the crayfish differ from that in the earthworm? Are the spaces between organs part of the coelom or not, and why?

Begin Fig. 5

7. Digestive system. ***A.*** *Identify:*

Mouth (between mandibles)
Esophagus (short tube ventral to stomach)
Stomach (box-like, thin-walled, with 2 transverse hardened bars)
Intestine (slender tube, dorsal in abdomen; posterior end in lobster is enlarged as rectum with a blind pouch or caecum attached)
Anus
Digestive glands or liver (soft, on either side, lateral to mandibular muscles; yellow-green in fresh specimen)

B. *Remove the stomach by cutting across the intestine and esophagus, taking care not to injure nerves around the latter. Open the stomach ventrally by a lengthwise cut, wash out any food within, and distinguish:*

Cardiac chamber (anterior, larger)
Teeth or gastric mill (1 median, 2 lateral, in cardiac chamber)
Filter (of fine hair-like setae between chambers)
Pyloric chamber (posterior, smaller)

Pull the bases of the stomach muscles to imitate the action of the teeth in grinding food. Why does an animal with pincers (chelae) and jaws (mandibles) also need a "gastric mill"?

8. Excretory system. In the head, just inside the base of each antenna, is the soft sac-like green gland with a duct leading to the excretory pore on the base (protopodite) of the antenna.

9. Reproductive system. The gonads in both sexes are paired but joined posteriorly to form ≻ -shaped masses.

A. MALE. Each of the 2 testes is a slender convoluted white tube and connects to a fine much-coiled vas deferens which opens on the base of the last pair of walking legs.

B. FEMALE. The ovary contains many small round yellowish eggs. From each ovary an oviduct connects ventrally to open on the third pair of walking legs.

Complete Fig. 5

10. Muscles. Each jointed part of the body and appendages is moved by opposed sets of muscles, as seen in the chela. In the abdomen these are conspicuously segmented, the smaller extensor muscles serving to straighten that part and the large flexor muscles to bend it in swimming.

Why do the two sets of abdominal muscles differ so greatly in size and power?

11. Nervous system. *Remove organs remaining in the thorax, noting the sternal artery that passes down to supply the ventral arteries of the thorax and abdomen; it passes through an opening in the nerve cord. Remove the muscles in the abdomen.* In the thorax the nerve cord lies in a ventral compartment (sternal blood sinus) below a series of chitinous plates (endophragmal skeleton); *cut these away and identify:*

Brain or supraesophageal ganglia (in head; with 3 pairs of nerves to eyes, antennules, and antennae)
Connectives (2; slender, around esophagus)
Subesophageal ganglia (with paired nerves to appendages about mouth)
Ventral nerve cord (double, lengthwise, with segmental ganglia giving off pairs of nerves to appendages and internal organs)

How many ganglia are present? What relation do they bear to the somites?

Draw Fig. 6

12. Sense organs. *A.* EYE. *Carefully slice one eye and its stalk in median section, place in water, and under low magnification distinguish on the cut surface:*

Cornea (thin external covering)
Ommatidia (small radial components of the compound eye)
Nerve ganglia (in stalk)

Slice off part of the outer surface of an eye, remove the pigment from the inner surface, and mount the transparent portion (cornea) *in water on a slide; add a coverglass. Under a microscope find the many rectangular facets, each of which formerly covered the outer end of an ommatidium.*

B. STATOCYST. An organ of equilibrium is located dorsally in the base (protopodite) of each antennule. *Remove this segment, cut off the ventral side, and find within fine grains of sand and sensory hairs.* How does this sensory structure function? How are the sand grains obtained?

Why is the crayfish supplied with such well-developed sensory organs in comparison with the earthworm and with other lower invertebrates? Why are the eyes on stalks? What types of vision are possible with compound eyes?

13. Other crustaceans. *Examine other types of the* Class CRUSTACEA *that may be exhibited.*

What part do crustaceans play in the cycles of life in fresh and salt waters? What are some conspicuous types used as human food? Are any crustaceans concerned in the life cycles of worm parasites? What are some types of parasitic crustaceans? Why are barnacles classified as crustaceans?

DRAWINGS

Fig. 1. Crayfish (150 mm. long), lateral view; outline the body parts and appendages. Label parts listed in Par. 1 that are visible.

Fig. 2. Appendages, of right side, in posterior view (each ×2); include (*a*) antenna, (*b*) mandible, (*c*) third maxilliped, (*d*) third walking leg, (*e*) second swimmeret.

Fig. 3. Cheliped (natural size), lateral view, as opened to show muscles in pincer.

Fig. 4. One gill (podobranch), lateral view (×3); label the parts.

Fig. 5. Internal organs (125 mm. long), dorsal view, in outline of body.

Fig. 6. Nervous system (125 mm. long), dorsal view. Number the ganglia of the nerve cord by the somites in which they occur and label parts named in Par. 11.

EXERCISE 39. GRASSHOPPER

Phylum **ARTHROPODA** Class *INSECTA*

(Storer, "General Zoology," pp. 460–471)

Like other arthropods, the insects possess segmented bodies, jointed appendages, and a chitinous exoskeleton; but as a Class they are distinguished by having one pair of antennae and a body of three conspicuous subdivisions—head, thorax, and abdo-

men. Typically the thorax bears three pairs of legs and two pairs of wings; some have only one pair of wings (DIPTERA, etc.), and others are wingless (fleas, ticks, and others). The insects are mostly terrestrial; they breathe air which enters small lateral openings (spiracles) on the body and circulates in a system of ducts (tracheae) to all organs and tissues. Their mouth parts are adapted either for chewing or sucking (text, Table 23·2), and they feed variously on plant or animal materials.

Insects for study collections are captured mainly with hand nets of fine-meshed cloth, killed in bottles containing potassium cyanide (KCN, a deadly poison), mounted on special slender pins, and kept in tight wooden boxes designed to exclude certain small beetles that will destroy the dried specimens. Large collections are stored in special museum cases and fumigated at intervals for protection. Immature stages of insects—larvae or nymphs—are preserved with alcohol or other fluids in small glass vials. Series of specimens showing the life cycles of individual species of insects and other special exhibits are often placed in shallow glass-topped boxes lined with cotton (Riker mounts).

Specimens for dissection are preserved in fluid and usually have the body injected with preservative to ensure fixation of the soft internal organs. For elementary study, a grasshopper (locust) serves well, being large, easily obtained, and somewhat generalized in structure. Species commonly used are the winged "Carolina locust" (*Dissosteira carolina*) or the "American locust" (*Schistocerca americana*), and the short-winged "lubber grasshopper" (*Romalea microptera*).

1. External features. *Examine an entire grasshopper, and identify the major subdivisions and parts of the body:*

HEAD	THORAX	ABDOMEN
Antennae (2; slender)	Prothorax (anterior)	Somites (number ?)
Compound eyes (2; large, lateral)	Mesothorax (middle)	Spiracles (small openings on sides of somites)
Ocelli or simple eyes (3; small, between compound eyes)	Metathorax (posterior)	Auditory organs (2; lateral, on first somite)
Mouth parts (ventral)	Legs (3 pairs)	Cerci (2; short dorsal spurs, near end of abdomen)
	Wings (2 pairs)	Ovipositor (on female, of 4 spurs)
		Anus

Draw Fig. 1

The body is covered with hardened plates or ***sclerites*** (without limy deposits); the softer parts or joints between sclerites are called ***sutures.*** The head lacks evidence of segmentation and is enclosed mostly by one fused sclerite (epicranium) on which the distinguishable regions are:

Vertex (dorsal) Gena (2; lateral) Frons (anterior)

Below the frons is a broad sclerite termed the ***clypeus.***

2. Mouth parts. *A. Examine a demonstration dissection or a prepared mount of the mouth parts. Then, working with forceps and under a lens or binocular microscope, remove and pin out the parts in their natural relations* (anterior side uppermost); *identify:*

Labrum or upper lip (1; broad; hinged to clypeus)
Mandibles or jaws (2; heavy, with teeth)
Maxillae (2; of several parts)
  Maxillary palp (one lateral on each maxilla)
Hypopharynx or tongue (1; slender, pear-shaped, median)
Labium or lower lip (2 fused as 1)
  Labial palps (one on either side of labium)

B. Identify the subdivisions of the maxilla and labium (text, Fig. 23·3).

Draw Fig. 2

C. Examine the mouth parts of any other types of insects that may be available. If live grasshoppers or other large, chewing insects are available, watch the action of the mouth parts. In what respects do the mouth parts of the grasshopper resemble and differ from those of the crayfish? How does the action of the jaws compare with those in a vertebrate—man, frog, etc.?

3. Wings. *Remove, spread, and pin out the fore and hind wings of the left side. Find the fine veins* (nervures), *especially in the hind wing. If a live cockroach is available, watch the blood circulation in the veins under a binocular microscope with strong illumination.*

Draw Fig. 3

How do the two wings of the grasshopper differ in structure? In function? How do the wings differ from those of other insects that may be demonstrated (see also text, Figs. 23·19–23·47)? How do they differ from those of a bird and of a bat (text, Fig. 9·1)? Which animal wings are analogous structures and which homologous?

4. Legs. *A. Carefully remove the three legs from the left side, using care to bring away the basal segment of each. Pin out in order, lateral surface uppermost. On each, identify from base to tip:*

Coxa
Trochanter
Femur
Tibia
Tarsus
  Claws (lateral)
  Pulvillus (pads; number?)

Draw Fig. 4

Is there a fundamental similarity between the three legs? In what respects do they differ from one another? How do they compare with the walking legs of a crayfish? With the legs of a frog or mammal? In what ways are they specialized for the manner of life of the grasshopper?

B. Examine legs of any other insects that may be demonstrated. Do they resemble the legs of the grasshopper, and if so, how? What differences are evident?

5. Internal structure. *With scissors, and beginning at the tip of the abdomen, make a lengthwise cut in the body covering slightly to the left of the middorsal line and along the entire length of the grasshopper. Make a similar cut ventrally and also up the front of the head. Keep the inner scissors point just inside the body covering to avoid damaging internal organs. Pin the grasshopper in water, right side down, and then carefully lift and dissect off the body covering of the left side. If the specimen is a mature female, the interior spaces may be filled largely with slender eggs in the ovaries; remove some of these if so directed by the instructor. Locate the following organ systems:*

Integument and exoskeleton	Digestive	Excretory
Muscular	Circulatory	Nervous
	Respiratory	Reproductive

Begin Fig. 5

6. Muscular system. *In studying other systems, note the many muscles, especially those in the thorax connecting to the legs and wings and those in the abdomen.* Why are the latter so much smaller than in the crayfish?

7. Digestive system. *Remove such of the lateral muscles and tracheae as necessary without injuring other organs; flood the specimen with water from a pipette to aid in distinguishing the organs. In the digestive system, identify:*

Mouth (between mandibles)
Salivary glands (slender, branched, whitish, ventral to crop)
Esophagus (slender, short, in head)
Crop (large, thin-walled, in thorax)
Gizzard or proventriculus (short, thick-walled)
Gastric caeca (6, double, on sides of stomach; function?)
Stomach or ventriculus
Intestine (tapered, in abdomen)
Rectum (swollen, near end of abdomen)
Anus

8. Circulatory system. The fine tubular ***heart*** is dorsal in the abdomen and connects to an ***anterior aorta.*** (These parts often disintegrate in preserved specimens.) Blood is pumped into the spaces between tissues, which comprise a hemocoel (not a true coelom), whence it returns to the heart.

9. Respiratory system. From the small ***spiracles*** opening laterally on the abdomen (and thorax), air enters the large lateral trunks of the system of ***tracheae*** and passes through smaller and smaller tubules, finally passing into the microscopic ***tracheoles*** that serve the tissues. *Mount part of a large tracheal vessel in water on a slide, press down under a coverglass, and examine under medium and high magnification. Find the spiral thread-like thickening of the lining* (cuticle) *that prevents collapse*

of the tracheae. Also make a water mount of muscle or other light-colored organ and note the distribution of tracheoles to the tissues.

By what method is air drawn into and forced from the tracheal system to serve in respiration? How does respiration in an insect differ from that in a crayfish? From that in a land vertebrate?

Draw Fig. 6

10. Excretory system. Many thread-like ***Malpighian tubules*** (excretory system) attach between the stomach and intestine. How are the organic wastes collected? How do they leave the body? How does excretion in an insect compare with that in a crayfish and in a frog?

11. Reproductive system. *Dissect the system under water; later examine that in a specimen of the opposite sex as dissected by some other student. Identify:*

MALE

Testes (2; dorsal, of many slender lobes)
Vasa deferentia (2; small slender ducts)
Ejaculatory duct (1; ventral, receives both vasa deferentia)
Accessory glands (several coiled tubes joining base of ejaculatory duct)
Copulatory organ (large, median, conical, at end of abdomen)

FEMALE

Ovaries (2; dorsal, of slender oblique tubes)
Oviducts (2; one lateral along each ovary)
Vagina (1; midventral, wide, below rectum; receives both oviducts)
Seminal receptacle (1; pear-shaped with long duct, dorsal on vagina)
Bursa copulatrix
Ovipositor (of 4 hooked processes at end of abdomen)
Egg guide (midventral)

Draw Fig. 7

12. Nervous system. As in the crayfish, this system in the grasshopper occupies the head and the ventral floor of the thorax and abdomen. *Carefully cut across the esophagus and rectum and remove the digestive tract. Remove muscles and portions of internal* (endophragmal) *skeleton in the floor of the thorax as needed to expose the nerve ganglia and cord. If the side of the head was not cut away previously, carefully dissect off the remaining exoskeleton to expose the brain. Identify:*

Brain or supraesophageal ganglia (3 pairs of closely fused ganglia, with nerves to the 3 ocelli and other parts and one large ganglion to each eye)
Connectives (2; slender, around esophagus)
Subesophageal ganglia (3 pairs, closely fused, ventral in head)
Ventral nerve cord (double, lengthwise, with segmental ganglia [fused pairs] giving off paired nerves to internal organs and appendages)
Symphathetic system (minute, often destroyed; of median and lateral connectives [nerves] from brain, with small ganglia, extending to crop, etc.)

Complete Fig. 5

How many ganglia are present in the ventral nerve cord? What relation do they bear to the body somites?

DRAWINGS

Fig. 1. Entire grasshopper (150 mm. long), lateral view; show and label parts named in Par. 1.

Fig. 2. Mouth parts (× 5), ventro-anterior view; show and label all parts identified.

Fig. 3. Fore and hind wings of one side (each × 3), spread, in dorsal view. Label margins.

Fig. 4. Legs of left side (each × 3), lateral view; show and label subdivisions of each.

Fig. 5. Internal structure of grasshopper (150 mm. long), lateral view; show and label parts named in Pars. 7, 8, 10–12.

Fig. 6. Tracheae. Portion of one large vessel (30 mm. in diameter) and also a small piece of muscle (enlarged) to show fine tracheoles.

Fig. 7. Reproductive system, male or female (75 mm. long), dorsal view; show and label parts named in Par. 11.

EXERCISE 40. AMPHIOXUS

Phylum **CHORDATA** Subphylum CEPHALOCHORDATA

(Storer, "General Zoology," pp. 541–544)

The small slender animal known as amphioxus (lancelet, Genus *Branchiostoma*, etc.) is of especial zoological interest because it is the lowest type showing clearly in the adult the fundamental characteristics of the Phylum CHORDATA. These features are (1) a dorsal hollow central nervous system, (2) paired lateral gill slits (branchial clefts) off the pharynx, and (3) a notochord as the axial skeleton. Amphioxus is marine; it burrows in the sandy bottom under warm shallow coastal waters, leaving only the anterior end exposed. At night it may come forth and swim, but falls on its side when swimming movements cease.

For study, young specimens (10 to 15 mm. long) are stained, cleared, and prepared as permanent whole mounts; larger individuals (to 50 mm. long) may be simply cleared and mounted on slides, when many structural parts become visible through the then semitransparent body. Stained cross sections will show other details and the cellular structure.

1. General features. *Study an entire specimen in lateral view under low and medium magnification* (do not use high power); *identify:*

Body (no distinct head; trunk long and slender; tail short, behind anus)

Fins (dorsal, ventral, and caudal; all median and supported by many rectangular fin rays)

Metapleural folds (2; ventro-lateral along most of trunk)

Myotomes or segmental muscles (many, along trunk and tail, <-shaped, with lengthwise fibers)

Myosepta (thin partitions separating myotomes)
Oral hood (at anterior end, ventral)
Oral or buccal cirri (slender, projecting from hood)
Vestibule (space within hood)
Velum (vertical membrane at posterior end of vestibule)
Mouth (aperture in velum)
Velar tentacles (delicate, thread-like, around mouth)
Wheel organ (ciliated areas in vestibule that produce a rotating effect in life)
Pharynx (behind mouth, long, high; sides with many narrow diagonal gill bars and slits)
Stomach (behind pharynx)
"Liver" (slender hollow lobe extending anteriorly from stomach)
Intestine (slender; through posterior part of body, in coelomic cavity)
Anus (ventral, on left side, near posterior end)
Atriopore (ventral, median, posterior to metapleural folds)
Gonads (if present; many pairs, rounded, at sides of pharynx)
Notochord (slender rod, entire length of body, dorsal to digestive tract; with vertical striations)
Nerve cord or neural tube (dorsal to notochord; marked by pigment in central canal)
Cerebral vesicle (slight anterior enlargement of nerve cord)
"Eyespot" (black pigment anteriorly in cerebral vesicle)
Olfactory pit (dorsal to cerebral vesicle)

Draw Fig. 1

2. Cross section. *Examine a stained cross section through the gill region under medium and high magnification; identify the organs and structures, then examine the cellular structure in each:*

INTEGUMENT

Epidermis (covers body; of one cell layer)
Dermis (connective tissue under epidermis)

SKELETAL SYSTEM

Notochord (cells large, contain vacuoles)
Connective tissue (sheaths around notochord and nerve cord; myosepta between muscles)
Gill bars

MUSCULAR SYSTEM

Myotomes (alternating on 2 sides of body)
Transverse muscles (in floor of atrium)

DIGESTIVE AND RESPIRATORY SYSTEMS

Pharynx (large cavity with gill bars laterally)
Gill bars (cut obliquely; each contains supporting rod and blood vessel; covered by epithelium, partly ciliated)
Hyperbranchial groove (middorsal in pharynx; cells very narrow)
Endostyle (midventral in pharynx; cells tall, ciliated)
Atrial cavity (space on either side between pharynx and body wall)
"Liver" (if shown; in one side of atrial cavity)

CIRCULATORY SYSTEM

Dorsal aortas (2; above pharynx)
Branchial vessels (1 in each gill bar)
Ventral aorta (1; below endostyle)

EXCRETORY SYSTEM

Nephridia (in small spaces [remnants of coelom] on each side of hyperbranchial groove)

NERVOUS SYSTEM

Nerve cord (dorsal to notochord and with central canal; lateral nerves may show)

REPRODUCTIVE SYSTEM

Gonads (in male, contain minute developing sperm, deeply stained; in female, include larger developing ova)

CAVITIES WITHIN BODY

Atrium
Dorsal coelom (paired; above atrium and separated from it by mesentery-like partitions)
Ventral coelom (beneath endostyle)
Coelomic canals (in primary gill bars; connect dorsal and ventral coelom)
Lymph spaces (part of circulatory system; especially the paired metapleural canals in the metapleural folds)

Draw Fig. 2

In what respects does amphioxus resemble a vertebrate? Wherein is it different? How do the relative positions of the nerve cord and digestive tract differ from those in the earthworm or crayfish? How does amphioxus obtain food? Through what organs and spaces does the water for respiration pass? How do mature gametes escape from the body? What are the characteristics of the Subphylum CEPHALOCHORDATA?

DRAWINGS

Fig. 1. Entire amphioxus (200 mm. long), from left side; show myotomes only on part of the tail; include and label all other structures identified.

Fig. 2. Cross section of amphioxus (125 mm. high); outline and label the structures identified, then show cellular detail in a small part of each.